## Studies on spontaneous and induced mutation

Mutation is one of the basic processes of life. It is the ultimate source of hereditary variability and as such an essential prerequisite for evolution. Yet in spite of the great achievements of radiation genetics in the last 20 years, the nature of the mutation process is still unknown. The work presented here is an attempt to approach this problem through a study of chemical substances affecting mutation rates. It includes one paper on spontaneous mutation which grew out of incidental observations made in the course of an experiment with chemical treatment. Drosophila melanogaster was used throughout as test animal.

The first substances to be tested for mutagenic capacity were carcinogenic hydrocarbons. The use of these substances was suggested through the theory, supported by a number of scientists, that cancer is due to mutation in somatic cells. The results of mutation tests failed to give evidence in favour of this theory, and work along these lines was dropped for the time being (1).

In the course of this work, a sex difference had been noted, mutation rates being higher in the X-chromosome of males than of females, independent of treatment. In a number of large-scale tests with untreated flies this difference in the incidence of spontaneous mutations in the sexes could be substantiated (2).

In 1940, Prof.A.J.Clark and Dr J.M.Robson from the Department of Pharmacology drew my attention to the pharmacological similarities between mustard gas and X-rays. We considered the possibility that mustard gas, like X-rays, might exercise an action on the chromosomes. In collaboration with Dr Robson, experiments were started to test this hypothesis. The results of/-

of these experiments, being subject to a security ban, could not be published during the war. They were submitted to the Ministry of Supply in two reports (W 3979 and W 11831) which were handed in on the 14th of March and the 4th of June 1942. Permission for publication has only recently been obtained. For this reason most of the publications concerning this work are still in press, apart from a few preliminary notes (3,4,5). The papers which are in press are here submitted as proof or in typescript.

Our data show that mustard gas is an extremely efficient mutagen, comparable in degree of mutagenic activity to high-energy radiation. Like X-rays, it causes sterility through interference with gametogenesis. It causes dominant lethality, chromosome re-arrangements, visible and recessive lethal mutations (6). Most experiments were carried out with doses which produced between 6 and 13% of sex-linked lethals, but in one experiment as many as 24% were obtained. Visible mutations were mainly of the types which had occurred previously after irradiation or spontaneously, and no specific action of the gas on particular loci was observed. The frequency of translocations in several experiments fell short of what would have been expected from a dose of X-rays producing the same frequency of recessive lethals.

A number of substances which either in their pharmacological action or their chemical structure are related to mustard gas were next tested for possible action on chromosomes and genes. Several of them, all belonging to the class of the so-called nitrogen- or sulphur-mustards, were shown to be as potent as mustard gas. In addition, allyl isothiocyanate behaved as a weak, but definite mutagen. Two tested substances, chloracetone and dichloracetone, possibly have a slight mutagenic action. Lewisite, picric acid and osmic acid gave negative results. These data were submitted to the Ministry of Supply on

Dec./-

Dec. 23rd 1943 (Report Y 18171). A full report is in press (7).

In addition to their pharmacological interest, these results open up a new means of analyzing the process of mutation. Work along these lines was carried out without the cooperation of Dr Robson. Attempts were made to induce somatic mutations through treatment of Drosophila embryos with mustard gas. A great number of apparent somatic mutations were obtained, but subsequent analysis revealed that most or all of them were in reality somatic crossovers (8). These tests therefore show a very strong influence of mustard gas on somatic crossing-over. At the same time they suggest, as has indeed been urged before by Stern, that also after X-radiation of embryos the apparent somatic mutations may in fact be the results of somatic crossing-over.

Particular interest attaches to the differences between the mutagenic action of radiation on the one hand, chemical substances on the other. One of these differences consists in the strikingly high percentage of mosaic individuals among the progeny of chemically treated males. Analysis of this phenomenon led to the assumption that mustard gas, unlike X-rays, may exercise a delayed action on the chromosomes (9). An extension of this work to a study of mosaics for sex-linked lethals lent further support to this hypothesis (10).

In several instances, segregations which did not seem to fit into any acknowledged scheme of chromosome behaviour were observed among the progeny of chemically treated flies. One such case was analyzed more closely and appears to have been due to an action of the treatment on the centromere of the X(11).

A summary of the results which so far have been obtained with chemical mutagens/-

mutagens has been compiled in cooperation with Dr J.M.Robson and Dr J.G.Carr and is now in press (12).

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# Tests of Carcinogenic Substances in Relation to the Production of Mutations in *Drosophila melanogaster*

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# XIII.—Tests of Carcinogenic Substances in Relation to the Production of Mutations in *Drosophila melanogaster*.

By **Charlotte Auerbach,** Ph.D., Institute of Animal Genetics, University of Edinburgh. *Communicated by* Dr A. W GREENWOOD.

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## THE PROBLEM.

THE theory has often been put forward that cancer is a somatic mutation. The changes which differentiate the cancerous cell from the normal are reproduced in many or all of the cells derived from the original malignant cell, *i.e.* they show the properties by which we define a mutation. Thus, to a geneticist, the possibility which most readily suggests itself is (1) that of a mutation in the genetic material of the somatic cell. Other possible mechanisms that might simulate the results of (1) are (2*a*) the introduction into the cell of a malignant virus; (2*b*) the "activation" of some such virus pre-existing in the cell in an inactive state, a situation which would imply mutation in the virus since the change becomes reproduced; (3) the production of some autocatalytic substance other than gene or virus, inducing malignant changes in the cell which contains a certain amount of it.

Possibility (2*a*) has been found to hold in a certain number of cases, *e.g.* the Rous sarcoma of the fowl. On the other hand, there is no reason to suppose that the same changes which can be brought about by a virus may not in other instances be the result of a mutation in the germinal material proper to the cell itself. If we visualise the virus as a free gene in the protoplasm (Muller, 1926), these two causes, (1) and (2), would not in the last analysis be intrinsically different.

The third possibility, that of an autocatalytic substance, appears to have least to recommend it. It seems difficult to conceive of a substance of this nature able to produce, in the same kind of individual, presumably containing cells with the same genotypes, tumours of such widely differing, self-perpetuating types as are known, and it is likewise difficult to conceive of the existence of many different substances of this kind, if we exclude

the possibility of their being genes (either chromosomal or virus genes), which of course are of manifold sort and capable of mutation. And certainly the self-perpetuating changes which sometimes supervene in a tumour that is long cultivated or transplanted, altering its capacity to take in certain hosts (Strong, 1926), must be conceived of as mutations. Thus, on the whole, the somatic mutation theory, in one form or another, seems to offer the most plausible explanation for the phenomena of cancer, and to be the one which requires the least in the way of additional hypotheses.

To design experimental tests to distinguish clearly between the various possibilities mentioned above is very difficult, and has been attempted but rarely and then without decisive results. For example, Curtis, Dunning, and Bullock (1933), and Dunning, Curtis, and Bullock (1937), proved that under the conditions of their experiments—numerous independent foci of irritation—the change of a normal cell into a malignant one is a random event like mutation, and they regarded this as evidence for the mutation theory of cancer. Their data, however, might be equally well interpreted on the other conceptions of cancer, so long as it is assumed that the change into a cancerous cell under the influence of the given stimulus is a threshold affair, and that the irritating agent at each focus has a given chance of rising above this threshold, independently of what happens at the other foci—as is in all probability the case.

The above authors also found that age had no enhancing effect on the production of these tumours other than by increasing the time of exposure, which again they regard as evidence favouring the mutation theory, but this result too would seem as likely under the other conceptions. Likewise, the well-known fact that the incidence of mammalian tumours increases with age is only of doubtful corroborative value for the mutation conception, inasmuch as the increase is very much steeper than would be expected on the mere basis of the increased time allowing more opportunity for the event to occur and so must, at least partly, be attributed to special effects of age itself. Somewhat similar special effects of age have been reported for mutation rate in the case of seeds of plants (Navashin, 1933; Stubbe, 1935), but not yet in animals.

The main support for the mutation theory of cancer is still to be found in the consideration, first pointed out by Muller (1927; see also 1937) for X-rays, that the agent which has so far been found most effective in producing mutations, namely high-energy radiation (such as X- and radium-rays), is effective in producing cancer also. We may here point out that modern work with ultra-violet light now seems to permit the

important extension of this parallelism in effect on mutations and cancer respectively to the latter agency. The effectiveness of ultra-violet light in causing mutations has been proved by Altenburg (1930) and later workers in *Drosophila*, and by Noethling and Stubbe (1934), Stadler and Sprague (1936), and others in plant material. As very recently shown by Stadler and Uber (1938), and by Muller and Mackenzie (1939; also Mackenzie and Muller, in press), the mutations produced by ultra-violet light, unlike those from higher energy radiation, are all or in the vast majority of cases mutations of individual genes, not mutations of whole chromosomes or chromosome sections. Thus if the cancer said to be produced by ultra-violet light is of mutational origin, we must conclude it to be caused by gene mutations—a conception better fitting the expectation of modern geneticists—rather than by the gross chromosome changes to which it has often been attributed.

In the case of ultra-violet radiation, there is an opportunity of obtaining further light on the correctness of the mutation theory of cancer by a study of the relative effectiveness of different wave-lengths in the production of cancers as compared with mutations. It has been found in the work on plant material (Stubbe and Noethling, 1937; Stadler and Uber, 1938; and others) that, when differences of penetration of the rays are allowed for, there is a maximum of mutation-producing efficiency in the ultra-violet in the region near 265 m$\mu$, which corresponds with a region of maximum absorption by nucleic acid, and therefore by chromosomes, and disagrees with the absorption maxima for proteins as well as for the great majority of other cell constituents. If a similar maximum could be found for the cancer-producing effect, this would constitute strong evidence in favour of the mutation theory of cancer. As yet, sufficiently exact studies of this question have not been made.

If now it could be shown that there is also a parallelism between the induction of mutations under the influence of chemical substances and the induction of malignant tumours by such substances, the mutation theory of cancer would gain important further support. Unfortunately, all attempts hitherto made to induce mutations by chemical means have given negative or indecisive results. None of the experiments published up to the time our work began has, however, been concerned with substances known to be effective in producing cancer. It has therefore seemed worth while to try the effect of carcinogenic substances on the mutation rate of *Drosophila melanogaster*, the most suitable animal for studying mutation. Such experiments had in fact been projected several years ago by Dr Alexander Weinstein, and independently by Muller and others, with these considerations in mind, but had not,

to our knowledge, ever been carried out on a sufficient scale for the results to be reported.

## Technique.

The object of the various techniques tried was to expose proliferating gonadic tissue to the action of powerful carcinogenic substances. After a preliminary experiment on a small scale, involving the feeding of carcinogenic substances, had given a negative result, injection was used on a large scale and in divers variations. It was supplemented by a small number of insertions of crystals into the abdomen. The substances used were (1) 1 : 2 : 5 : 6-dibenzanthracene in 0·03 per cent. colloidal solution, (2) 9 : 10-dimethyl-1 : 2-benzanthracene in 0·06 per cent. colloidal solution, both suspended in a medium consisting of Ringer's solution as given by Ephrussi and Beadle (1936) minus calcium plus 0·5 per cent. gelatine. This medium by itself was used for the controls. The third substance, methyl-cholanthrene, was introduced in crystal form.

Injections were done by means of an ordinary pipette drawn out into a fine capillary and sharpened at its end in the way described by Ephrussi and Beadle (1936). Adults as well as larvæ were injected. The latter proved by far the more difficult. This was because the high viscosity of the colloidal solutions and their tendency to coagulate required the capillary to be so wide and the pressure used in injection to be so great that the majority of the injected larvæ died through subsequent oozing or infection of the wound. Injection of adults, on the other hand, was found to be extremely easy. Wounds, even when comparatively large, heal readily, and consequently the mortality is very low. The only danger appears to be an excess of fluid in the abdomen. The border-line between a dose mechanically tolerated and a lethal or sublethal one seems very distinct: whereas amounts between 0·2 and 0·3 c.mm. were easily tolerated (3rd and 4th series), amounts between 0·3 and 0·4 c.mm. killed 50 per cent. of experimental as well as control flies (5th and 6th series), and 0·5 c.mm. were almost lethal (2nd series). The amount of fluid injected was estimated from the diameter of the drop when an equal amount was suspended from the point of the pipette over a micrometer scale. Checks with the micro-burette of Fox-Wingfield showed this rough estimate to be sufficiently accurate.

Experimental and control flies were injected with the same amount of fluid and in the same spot. Injections of larvæ were done dorsally or laterally near the site of the gonads. Adults were injected either mid-dorsally or laterally after etherising and clipping of the wings. The medium was found to contain a considerable bacterial flora after only

two days, and the injections were then fatal to the flies, so that the medium for the control series had to be made up fresh for every injected batch. Medium containing carcinogenic substances, however, remained free from this type of noxious bacteria for many weeks, and in the first series was used repeatedly with intervals up to a fortnight between injections. But in order to avoid the suspicion that some undetected type of bacteria affecting the carcinogenic substances might have been present in the older solutions, series 5 and 6 were carried out with solutions made up on the day of the injection.

Crystals were inserted into adult females mid-dorsally behind the fourth abdominal ring. With a blunt glass needle they were pushed into a hole pierced previously by a pointed glass needle. Their size varied considerably; the majority were thin needles about 0·5 mm. long and 0·05 mm. in diameter. Dissections of some of the treated flies after a few days showed the crystal between the ovaries, sometimes even touching one of them.

Injected larvæ were allowed to pupate on moist filter-paper, and collecting of offspring was carried out from the hatching of the imago to its death. Injected males were kept with other females for a fortnight before mating them to the females whose offspring were used for the test. This was to induce active functioning of the testes and ensure the test being conducted on gonadic tissue in a proliferating condition at the time of treatment. Similarly, the collection of eggs from injected females was started 10–14 days after injection, during which interim they were kept with males, and the collection was continued up to 50 days. Treated females were divided into several batches and kept under differing conditions of temperature and food, a parallel series of control females being kept for each set of conditions. Control flies were taken from the same stock, and, when possible, from the same culture as the experimental flies. Care was taken to keep the number of treated and control animals in each group reasonably high in order to exclude the possibility that the results might be falsified by the presence of individual differences in the spontaneous mutation rate

The incidence of sex-linked lethals was taken as an estimate of mutation rate. Mutations occurring in the males were detected by the usual ClB method. Females to be injected were heterozygous for sex-linked markers and for inversions preventing the appearance of cross-overs, so that their sex chromosomes could be recovered intact from their daughters, when the latter were tested for the presence of sex-linked mutations received from the treated generation. The presence of a sex-linked lethal in an injected female antecedent to treatment was avoided in each case by

determining that she produced both expected types of sons. The genetic formulæ of the crosses are shown below.

$P_1$ female $\frac{sc^8 w^a bb}{sc^8 w^a bb}$ × male $sc\,\delta 49\ v\ od\ ca$.

$F_1$ female $\frac{sc^8 w^a bb}{sc\,\delta 49\ v\ od\ ca}$ (injected) × male $v B^{M1}$.

$F_2$ two types of females:

(1) $\frac{sc^8 w^a bb}{v B^{M1}}$ (appearance moderate bar eyes).

(2) $\frac{sc\,\delta 49\ v\ od\ ca}{v B^{M1}}$ (appearance vermilion eyes, moderate bar).

$sc^8$ = scute 8, bristles missing; long inversion.
$w^a$ = apricot, eye colour.
$bb$ = bobbed, bristle shape.
$B^{M1}$ = Bar M1, eye shape; inversion extending from B to "inert region."
$v$ = vermilion, eye colour.
$od$ = outstretched, wings.
$ca$ = carnation, eye colour.
$\delta 49$ = inversion of moderate size in middle of X.

The $F_2$ females were mated in separate vials to brothers, and their progeny examined for $w^a$ sons in the case of type $(1)_{♀♀}$, and for *od* sons in the case of type $(2)_{♀♀}$. The absence of such sons was taken as evidence of a lethal in the X-chromosome of the mother, *i.e.* the $F_2$ female. Since this X-chromosome came from the treated $F_1$ female, the lethal must have arisen in one of her germ cells through mutation. Thus the number of fertile $F_2$ cultures can be taken to represent the total number of tested chromosomes of the $F_1$ female, and the number of $F_2$ cultures with a lethal to represent the number of lethals which have arisen among these chromosomes.

## Results.

*1st Series* (preliminary).—1 : 2 : 5 : 6-dibenzanthracene. Females. Injections given laterally, into or very near the ovary, alternately left and right, from 2 to 6 times, with several days interval between injections. Total amount of substance injected ranging from about 0·25 c.mm. to 0·75 c.mm., corresponding to 0·08 $\gamma$ up to 0·24 $\gamma$ of substance. No controls. No lethals among 241 chromosomes tested.

*2nd Series.*—1 : 2 : 5 : 6-dibenzanthracene. Females. One mediodorsal injection of 0·5 c.mm., corresponding to 0·15 $\gamma$ of substance. Of 59 injected females only 4 survived. No lethal in 382 chromosomes, and no lethal in 265 control chromosomes.

*3rd Series.*—9 : 10-dimethyl-1 : 2-benzanthracene. Females. Treatment once (83 females surviving) or twice (46 females) with medio-dorsal or simultaneous right and left injections, each time 0·2–0·3 c.mm., corresponding to about 0·15 $\gamma$–0·2 $\gamma$. Various sub-series in respect of conditions following injection. 3 lethals, all from separate females, among 2919 examined X-chromosomes. Controls: 1 lethal among 1132 chromosomes, *i.e.* sensibly the same mutation rate.

*4th Series.*—9 : 10-dimethyl-1 : 2-benzanthracene. Males. One medio-dorsal injection of 0·2–0·3 c.mm. containing 0·15 $\gamma$–0·2 $\gamma$ of substance. 15 treated, 19 control males. 3 lethals, all from different males, among 685 tested chromosomes. Controls: 2 lethals from different males, among 445 chromosomes, *i.e.* no difference between treated flies and controls.

*5th Series.*—Repetition of third series with media made up the day of injection. 9 : 10-dimethyl-1 : 2-benzanthracene, injection once medio-dorsally with 0·3 c.mm., corresponding to 0·2 $\gamma$ of substance. The amount of fluid seemed to be near the threshold of mechanical toleration, since of 61 treated females and 56 control females only 25 survived in each batch, 19 of which were used for testing. No lethal was detected in 934 treated and 867 control chromosomes.

*6th Series.*—Repetition of fourth series with fresh media. 9 : 10-dimethyl-1 : 2-benzanthracene, 0·3 c.mm., corresponding to 0·2 $\gamma$ of substance injected once medio-dorsally. 26 survivors out of 60 treated males, and 27 out of 57 controls. 20 used in each batch for producing offspring. In 1125 treated chromosomes there were 8 lethals, 7 of these from different paternal chromosomes. In 814 control chromosomes there were 7 lethals, 5 from separate males. No difference in mutation rate was observed.

*7th Series.*—Methyl-cholanthrene. Crystals inserted into adult females. 12 females treated. 1 lethal in 734 chromosomes. No controls, but as the mutation rate appears as low as commonly found in untreated material, further testing of this method was not considered worth while.

*8th Series.*—9 : 10-dimethyl-1 : 2-benzanthracene. 9 female larvæ injected with about 0·3 c.mm., corresponding to 0·2 $\gamma$ of substance. No lethal in 749 tested chromosomes.

## Discussion.

None of the substances and techniques used gave a detectable increase in mutation rate over the controls. The amount which could be injected was of course very small, somewhat less than 0·2 $\gamma$ on the average. But as the average weight of *Drosophila* is not over 1 mg., the amount of

injected substance taken in proportion to body-weight corresponds to 40 mg. for a rat of 200 g., a dose which in the rat is more than sufficient for tumour induction with any of these substances. Conceivably, however, in the rat the substance will, by precipitation or otherwise, become concentrated in a relatively small area so that a direct comparison of the injected amounts taken in relation to the body-weights might not be feasible. A second reason for the failure to cause germ-cell mutations in the fly may be that an insufficient time was allowed for action, as offspring from treated flies could be collected for several weeks only, whereas in the rat several months are usually required for the detection of the tumour (the time of detection, however, being considerably later than the time of the primary cellular change). Other possible causes for the negative result are difficulties of penetration through the thin mesodermal membrane surrounding the ovarioles or through the even thicker testis sheath, differences in susceptibility between germ cells and somatic cells or between mammalian and insect cells, and differences in other factors, of an anatomical or chemical nature, between mammal and insect.

According to a paper by Sacharov (1938), which appeared after our own work had begun, this investigator obtained an apparently significant increase in mutation rate (18 lethals in 2921 treated, or 0·62 per cent. as compared with 87 lethals in 33,975 control chromosomes, or 0·25 per cent.) by treating *Drosophila* eggs with a solution of methylcholanthrene. Since, however, differences in mutation rate of this order may arise spontaneously from unknown causes when genetical and environmental conditions have not been very exactly controlled, and since similar differences are reported in the same paper for a considerable number of treatments with different chemicals, it does not seem possible to assess the importance of this result before the details concerning the conduct of the experiment are published. It would appear, however, to give some encouragement to further attempts along these lines, using varying types of technique.

An incidental result of the present study was the pronounced sex difference in spontaneous mutation rate in the stocks used (compare series 3 with 4, and 5 with 6). Experiments to follow up this rather surprising finding have been started.

## Summary.

Attempts to increase the mutation rate in *Drosophila melanogaster* by introduction into the body of carcinogenic substances as colloidal solutions or crystals have given a negative result.

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# THE EFFECT OF SEX ON THE SPONTANEOUS MUTATION RATE IN *DROSOPHILA MELANOGASTER*

BY

CHARLOTTE AUERBACH

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# THE EFFECT OF SEX ON THE SPONTANEOUS MUTATION RATE IN *DROSOPHILA MELANOGASTER*

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## Introduction

In experiments designed to test the influence of carcinogenic substances on the mutation rate in *Drosophila melanogaster* (1940), pronounced differences were observed between the rates with which sex-linked lethals arose spontaneously in male and female germ cells. The flies used for testing the mutation rate were $F_1$'s from crosses between females of a $sc^8 w^a bb$ stock and males of a $sc v \delta 49 od ca$ stock. When only the lethals arising in the $sc^8 w^a bb$ chromosome are considered the figures are: no lethal in 846 chromosomes derived from females, and twenty-seven lethals (from twenty-four different individuals) in 3771 chromosomes derived from males. These results had been guarded against any error due to the occurrence of non-disjunction by the scheme of crossing used. Results pointing in the same direction had been reported by Muller & Altenburg as far back as 1919, but as the differences then observed were not statistically significant, and as significant data were difficult to obtain with the low natural mutation rate soon afterwards found in the stocks used, these indications were not followed up. The present differences, however, were so striking that further investigations into this problem of fundamental importance appeared promising.

## Experiment I

Following the above preliminary results, the first test was carried out with the $sc^8 w^a bb$ stock which had been used as one of the parental stocks in the experiments on carcinogenic substances. Males and females to be tested for the occurrence of sex-linked lethals in their germ cells were taken from the same stock bottles. The males were tested by the usual *ClB* method, the females by means of the following scheme of crosses:

$P_1$ ♀ $\frac{sc^8 w^a bb}{sc^8 w^a bb} \times$ ♂ $sc v \delta 49 od ca$ (pair-matings, 23 pairs);

$F_1$ ♀ $\frac{sc^8 w^a bb}{sc v \delta 49 od ca} \times$ brother $sc^8 w^a bb$ (pair-matings, 30–60 pairs from each $P_1$ ♀).

In the absence of a sex-linked lethal the $F_2$ males consist of two types which are readily distinguished through the glass wall of the culture vial. If a lethal arises in the germ track of a $P_1$ female, one of her daughters fails to produce $w^a$ sons, the production of cross-overs by the $F_1$ being prevented by the presence of different inversions in the two $X$-chromosomes. By mating the $P_1$ in pairs any lethal already present in a $P_1$ female could be detected by the low sex ratio of her offspring, and such females were excluded from the test. Likewise excluded were $P_1$ females which produced non-disjunctional *sc v δ49 od ca* sons, because the appearance by secondary non-disjunction of $w^a$ $F_2$ males might mask the presence of a lethal on the $w^a$ chromosome. There still remain the possibilities of primary non-disjunction in the $F_1$, which in the presence of several inversions cannot be neglected, and of an extra $Y$ introduced from the $P_1$ male causing secondary non-disjunction in the $F_1$. The precaution against these sources of error in experiment I was not to classify any $F_2$ progeny as lethal-free unless at least three $w^a$ males were found on superficial inspection through the glass of the vial, and in doubtful cases to rear an $F_3$. This seems sufficient to exclude cases of primary non-disjunction, but some cases of secondary non-disjunction due to the presence of an extra $Y$ in the $P_1$ male may have remained undetected and create a source of error which is not altogether negligible.

The results were as follows: no lethal in 843 chromosomes derived from females; five lethals and one semilethal (one male among more than fifty females) in 538 chromosomes derived from males.

### Experiments II and III

In order to eliminate differences of genotype—apart from those necessarily existing between the sexes—the following tests were carried out with males and females from Florida wild-type stocks made isogenic by Singh through a sequence of crosses described in his thesis (1940). Two of these stocks were used: "Florida 4" and "Florida 5" (*Fo* 4 and *Fo* 5). As in each of these stocks by far the greater part of the major chromosomes of every individual is derived from one and the same ancestral haploid set, these flies constitute a nearly homogeneous material in respect of genotype—barring, of course, new mutations which may have arisen between the time the stocks were completed and the beginning of our experiments. At the same time, environmental differences between the flies under test were reduced to a minimum by rearing them under controlled and as nearly as possible identical conditions of food, temperature, and moisture, by taking $P_1$ males and females from the same

bottles in approximately the same numbers, and by randomizing the unavoidable individual differences between $P_1$ individuals through the use of a fairly high number of $P_1$ couples in each series. Males were again tested by the *ClB* method, females were tested by the following crosses:

"$P_0$"[1] ♀ $\frac{Fo}{Fo} \times$ ♂ *Fo* (controlled and identical conditions);

$P_1$ ♀ $\frac{Fo}{Fo} \times$ ♂ $sc^{S1}Lw^a sc^4R$ (23 pairs in experiment II, 37 pairs in experiment III);

$F_1$ ♀ $\frac{Fo}{sc^{S1}w^a sc^4} \times$ brother *Fo*.

A lethal in the *Fo* chromosome becomes apparent by the absence of wild-type males in $F_2$. As before, precaution was taken against lethals present from the start, and against secondary non-disjunction due to a *Y*-chromosome introduced from a $P_1$ female. Moreover, in each batch of $F_1$ females derived from a $P_1$ pair, a number of females were mated as virgins to $yw^a B$ males. If a *Y*-chromosome had been handed on from the father, some of these females would be expected to produce sons of paternal type, and in this case the whole batch was discarded. By accepting as lethal-free only those $F_2$ progenies in which at least three wild-type males were observed through the glass of the vial and by subjecting the doubtful cultures to further breeding tests, precaution was taken against occurrences both of primary non-disjunction in cells of the $F_1$ ♀ and of double crossing-over between the two *X*-chromosomes of the $F_1$ female. The results were as follows:

Experiment II. No lethal in 815 chromosomes derived from *Fo* 5 females. Nine lethals (from six different males) in 841 chromosomes derived from *Fo* 5 males.

Experiment III. No lethal in 796 chromosomes derived from *Fo* 4 females. One lethal and one semilethal (three males among more than seventy females) in 790 chromosomes derived from *Fo* 4 males.

## EXPERIMENT IV

The data of experiment III, though not disproving the earlier results, yet do not confirm them. It was therefore deemed desirable to test the question again on a larger scale. One more experiment was carried out, using *Fo* 5. The technique was the same as before except for four alterations: (1) Special care was taken to test germ cells of young individuals

[1] "$P_0$" is used to designate the generation preceding that of the flies ("$P_1$") whose mutation frequency was tested.

only, by mating the $P_1$ flies a few days after collection, keeping them on syrup food between collection and mating, and removing them from the vials after 3–4 days. (2) $sc^{S1}Lw^{a}In\text{-}Ssc^{8}R$ males that had been made up for such purposes by Muller were used for the $P_1$ instead of the rather inviable $sc^{S1}w^{a}sc^{4}$ males. The presence of inversion $S$ in the middle of the $X$-chromosome (Muller, 1935) renders the suppression of cross-overs complete. (3) In the later part of the experiments, the $F_1$ females were mated to $y^{2}sc^{8}w^{a}B$ males. Though the females were not virgins, a sufficient number of $B$ daughters were usually produced to allow an easy decision whether the absence of $w^{a}$ males in certain $F_2$ progenies was due to a lethal in the $sc^{8}w^{a}In\text{-}Ssc^{S1}$ chromosome or to the mother ($F_1$) having been a homozygous wild-type ♀ derived by primary non-disjunction in the $P_1$ female or by her non-virginity; thus simultaneous observation of lethals in both chromosomes could be carried out with only a little more labour. (4) For detecting an extra $Y$ in the $P_1$ males each male was tested by mating it to a virgin female carrying $bw^{v\text{-}A}\ Bl\,L^{2}$. The presence of an extra $Y$ is easily discovered in the offspring by the appearance of a number of non-*Bl* non-$L^{2}$ flies in which the mottling of the eye has been suppressed. All daughters of $P_1$ males with extra $Y$'s were excluded from the test.

The results of experiment IV were as follows: three lethals (two of them from the same female) and one semilethal (two wild-type males) in 2744 chromosomes derived from *Fo* 5 females; fifteen lethals and one semilethal (five males among over fifty females) in 2691 chromosomes derived from *Fo* males. In addition, twelve lethals (from eight different $P_1$ males) were found among the 2744 $sc^{S1}LIn\text{-}Sw^{a}sc^{8}R$ paternally derived chromosomes in the series in which the maternally derived *Fo* chromosomes were being tested. This latter finding may be taken as to some degree confirmatory of the relatively high mutability of the $X$-chromosome in the male, although of course the flies supplying this $w^{a}$-containing chromosome were genetically different from those of *Fo* 5.

When the data, as tabulated in Table 1, are pooled according to the method developed by Muller (1940)—disregarding the semilethals, and in the male series counting as separate only mutations which arose in different males—the difference in the percentage of sex-linked lethals turns out to be 0·48 % with a standard error of 0·11 %. As the difference is 4·4 times its standard error the result is statistically well secured. Analysis of the data gained in this experiment showed that the apparent discrepancy of the results gained in experiment III from the rest was in all probability only a result of "accidental" circumstances: in experi-

ment IV, also, there occurred one run of over 600 $F_2$ families without a single lethal.

Table 1. *Summary of experiments I–IV*

| | | Chromosome derived from female | | | | Chromosome derived from male | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. of experiment | Chromosome tested | No. of fertile $F_1$ cultures | No. of lethals (in brackets: semilethals) | From how many different ♀♀ | Percentage of lethals | No. of fertile $F_1$ cultures | No. of lethals (in brackets: semilethals) | From how many different ♂♂ | Percentage of lethals |
| I | *sc*$^8$ *w*$^a$ *bb* | 843 | 0 | — | 0 | 538 | 5 (+1) | 6 | 0·93 |
| II | *Fo* 5 | 815 | 0 | — | 0 | 841 | 9 | 6 | 1·07 |
| III | *Fo* 4 | 796 | 0 | — | 0 | 790 | 1 (+1) | 2 | 0·13 |
| IV | *Fo* 5 | 2744 | 3 (+1) | 3 | 0·15 | 2691 | 15 (+1) | 16 | 0·56 |

## DISCUSSION

The data presented above appear to establish a difference in the rate at which sex-linked lethals arise spontaneously in the sexes, the males having the higher mutation rate. From what we know about the different types of mutation, there is no reason to suspect that this sex difference should not extend to viable and autosomal gene mutations as well. As to its causes, only assumptions can be put forward as yet. If subsequently it should become possible to decide between them experimentally, this might bring us one step nearer the truth about the origin of natural mutations.

In their qualitative gene content, males and females of an isogenic stock differ only by the presence of the *Y*-chromosome in the former. It does not seem very likely that the *Y*-chromosome should influence the occurrence of mutations. Females carrying a *Y*-chromosome or a portion of it might be used to test this possibility.

When the mutations for which we test are sex-linked lethals, the possible occurrence of germinal selection in the male but not in the female has to be taken into account. Its effect would be to reduce the number of observable lethals in the male. If, therefore, it had occurred in the present experiments to any considerable degree, the observed difference between males and females would assume even more significance.

A possible difference between the sexes which might be considered as underlying the observed difference in mutation rate is one in respect of the number of cell divisions intervening between the fertilized egg that is to develop into the $P_1$ and that of the next generation ($F_1$), in which the mutant gene is found to have been present. If this number were considerably higher in the male, and if mutation occurred exclusively or mainly during the process of reduplication of the genes (a possibility

tentatively suggested by Muller, 1928, and apparently supported by results of Olenov, 1939, and of Singh, 1940), a superiority of the male in respect of mutation rate would be expected. Both assumptions, however, are as yet unproved. Muller's suggestion would find support if it could be shown that correlated with the higher frequency of mutants in the *Drosophila* sperm as compared with the egg was a markedly greater number of mitoses during its life history. Unfortunately, the proof for this is not easy to adduce, though at first sight one would suppose that the larger number of spermatozoa would require a larger number of preceding divisions. To arrive at a rough idea of the number of mitoses between fertilized egg and mature reproductive cell in either sex, the following calculations can be made, taking the female first.

According to Huettner (1923), the polar cells are differentiated from the blastoderm cells at the 256 nuclei stage, i.e. after eight previous divisions. There are five to eleven of them, and they form an average of fifty egg strings (ovarioles) in the mature female (Donald & Lamy, 1937). To obtain fifty initial cells for the fifty egg strings from eight to ten pole cells two to three mitoses are required. The total output in eggs of a *D. melanogaster* female averages about 1000, i.e. about twenty eggs per ovariole. Assuming that twenty oögonia are formed in the end filament as forerunners of the twenty eggs to be produced, and that these twenty oögonia are formed by simple dichotomous division, the numbers of cells after each subsequent division proceeding as the powers of two, four to five oögonial divisions have to be postulated. Almost certainly this figure is too low: if oögenesis followed this system no cells would be left in store at all. One division at least has to be set aside for the purpose of providing a store. Possibly there is considerably more storing. Also, there is no reason to assume that oögonial division always or mostly follows a dichotomous scheme. Certain mitoses may result in two cells, one of which only would go on dividing, the other being kept in store (or possibly becoming non-germinal). The extreme case of this type would be a division scheme in which one apical cell gives off one oögonial cell at a time, all oögonial cells being direct progeny of this apical cell. If, then, the oögonia developed directly into the egg, the first egg to be formed would require one oögonial division, the second two, etc. Twenty divisions would precede the formation of the twentieth egg, and ten divisions would be the average for all eggs formed during the lifetime of the fly. For the first eggs, however, which alone were used in experiment IV, the average would be much lower, perhaps two or three. If we allow each oögonium two more divisions before reaching the oöcyte stage the figure is raised to

four or five, i.e. the same as assumed above for a purely dichotomous mode of oögenesis. Next come four divisions producing the fifteen nurse cells and the egg proper, and finally the two oöcyte divisions. Adding up, we arrive at an estimate of $8+3+5+4+2=22$ mitoses preceding the formation of the mature egg.

In the male-forming egg development up to the formation of the polar cells is the same as in the female-forming egg, i.e. eight initial mitoses have to postulated. The five to eleven polar cells thus formed produce the two testes, which together, according to Kaufmann (oral report from Dr Koller), contain 8000–10,000 completed spermatozoa in the newly hatched male. In order to produce 10,000 spermatozoa from, say, ten initial cells by pure dichotomy, ten divisions (including the two spermatocyte divisions) are required. With an exclusively apical cell scheme of division, one primary cell in each testis would have to give off the 1000–1250 primary spermatocytes necessary to produce 4000–5000 spermatozoa. The number of mitoses preceding the primary spermatocytes would thus range from one for the first to at least 1000 for the last, with an average at 500–625. The spermatocytes then undergo two more divisions. Whereas the apical division scheme allows of a continuous formation of spermatogonia for the subsequent production of spermatocytes, the dichotomous scheme requires some previous storing (say four to five divisions in analogy to the estimate for the female). Adding up, we arrive at a minimum of $8+10+4=22$, and a maximum of $8+500+2=510$ or more mitoses preceding the formation of the sperm in the newly hatched male.

It will be seen that the minimum estimates do not differ for the two sexes. The maximum estimates, on the other hand, differ considerably. It is, however, almost certain that the pure apical scheme is not realized in spermatogenesis. Not only do the results gained by Harris (1929) provide evidence of at least two apical cells in each testis, but also cytological evidence on mitoses in the testes and on the time required is in contradiction to rigid apical proliferation. Most probably, actual spermatogenesis follows a system combining both modes of division. It can be seen that figures to fit any ratio between the two sexes could easily be made up by supposing a suitable intermediate between the two extreme schemes of gametogenesis. However, these remain mere speculations until independent information concerning gametogenesis has been gained.

Harris (1929), arguing from the fact that a mutation produced by X-rays 2–3 weeks previous to mating occurs in one-quarter of the sperm,

comes to the conclusion that "the proliferation of germ cells in the testis probably occurs through a system of one or a very few indefinitely reproducing cells functioning like apical cells". His data are, in fact, reconcilable with any other system of division as long as it is assumed that the sperm used in the late mating goes back to two spermatogonial cells present at the time of raying, and only if the situation as found by Harris after a definite time interval between raying and mating were true in general would it imply the existence of apical cells. Experiments of the same kind as those carried out by Harris, with raying at different ages, and checking on group formation of lethals at intervals of a few days, might, as Muller suggests, help to narrow down the scope of possibilities. Before more evidence on the method of gametogenesis is available, all that can be said in respect of its bearing on the sex difference in mutation rate is that it may conceivably be explained by a corresponding difference in the number of mitoses (including gene reduplications) during gametogenesis.

If we discard this explanation as unfounded, there still remains the possibility that the higher mutation rate in the male is an effect of some other physiological difference between the sexes. It is known for example that the catabolic processes differ between the sexes in many animals, the males having the higher catabolic rate. The hypothesis that metabolic processes should be able to influence mutation rate does not appear too fantastic in view of the influence on mutation rate found to be exercised certainly by temperature (Muller & Altenburg, 1919; Muller, 1928; Plough & Ives, 1932, 1935; Promptov, 1934; Timoféeff-Ressovsky, 1935; Buchmann & Timoféeff-Ressovsky, 1935, 1936; Zuitin, 1937, 1938*a*, *b*), and possibly by certain chemicals (Sacharov, 1932, 1933, 1935, 1936, 1938; Lobashov & Smirnov, 1934; Lobashov, 1935; Magrzhikovskaja, 1936, 1938), and by nutrition (Döring, 1937; Stubbe & Döring, 1938; Olenov, 1939). Higher rates of oxidation might influence the mutation rate through direct chemical effects or indirectly through influencing the nature of the medium in which the nuclei exist. If this explanation of the observed differences in germinal mutation rate were true, one would expect to find a corresponding difference in respect of somatic mutations. A higher rate of somatic mutations in the male could not be explained by a greater number of preceding mitoses; an influence of the *Y*-chromosome might be regarded as responsible, but could easily be tested in *XXY* females. Another test of the explanation by such differences in metabolism would consist in direct studies of the influence of altered metabolic rates on the occurrence of mutations. Studies of this kind are now in progress at this institute.

Incidentally our data also provide new evidence of the considerable amount of fluctuation of unkown origin in mutation rate already commented upon by others (cf. Muller, 1928), and it is notable in our work that this applies even to material very strictly controlled for genetical and environmental uniformity. Observations like this should serve still further to caution investigators working on spontaneous mutation rates or using them for control data. Reliable figures for spontaneous mutation rate can only be expected by using devices for maintaining such uniformity, by randomizing the remaining variations through the use of a fairly large number of parents, by taking precautions against sources of error through non-disjunction, crossing-over and the like and, above all, by working with sufficiently large numbers.

## SUMMARY

The spontaneous mutation rate in the two sexes was studied in flies from various stocks, mainly isogenic wild-type, reared under controlled and identical conditions. It was found to be markedly higher in the male, the difference being statistically significant. Fluctuations were considerable, even within the same experiment, and point to the necessity for strictest control of all conditions when gaining data on spontaneous mutations. Possible explanations for the observed results are discussed, but without further evidence along other lines no decision between them appears possible.

## ACKNOWLEDGEMENTS

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# Production of Mutations by Allyl *Iso*thiocyanate

IN the course of the past few years, we have examined a number of chemical substances for their ability to produce gene mutations. The experiments were carried out on *Drosophila melanogaster*. Some of the substances were found to be highly effective, producing mutation-rates of the same order as those obtained with X-rays, 6–24 per cent sex-linked lethals developing in treated *X*-chromosomes. These data will be published later.

Although the production of mutations by these potent synthetic substances is of great interest for the light it may throw on the nature of the gene and the process of mutation, the search for naturally occurring substances with the capacity to produce the same effect appears, from the point of view of evolutionary theory, even more important. It is therefore of special interest that among the substances tested we have found one, namely, allyl *iso*thiocyanate (mustard oil), which has a definite though slight effect on the mutation-rate, and which occurs naturally in a variety of plants, for example, *Brassica nigra* and other Cruciferæ (Klein[1]). A summary of the data on which this conclusion is based is given below. A full report will appear later.

The technique used was the *ClB* test for sex-linked lethals, which is the standard test used for detecting lethal mutations which develop in the *X*-chromosomes of the spermatozoa in treated (and control) males. Two experiments were carried out. With the second a control was done simultaneously on flies collected from the same culture bottles as the flies for treatment. The results are shown in the accompanying table.

| Expt. | No. of *X*-chromosomes tested | No. of lethals detected in the chromosomes | Lethals (per cent) |
|---|---|---|---|
| 1 | 756 | 17 (+1 doubtful) | 2·2 |
| 2 | 878 | 19 | 2·2 |
| Control | 963 | 4 | 0·4 } Diff. = 15±4·8 |

The difference between the treated and control series in the second experiment is clearly significant. Moreover, in both experiments the mutation-rate markedly exceeds the range of the spontaneous occurrence of sex-linked lethals in normal stocks, which scarcely ever reaches even 1 per cent.

In addition, three sex-linked visible mutations were obtained in the two treated series, none in the controls.

Experiments are under way to determine whether allyl *iso*thiocyanate can also produce chromosome breaks.

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[1] Klein, G., "Handbuch der Pflanzenanalyse", Part 2, Chapter 26 (Springer, Vienna, 1932).

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## Chemical Production of Mutations

IN a previous letter in *Nature*[1], chemical substances were mentioned which are as effective as X-rays in inducing mutations and chromosome rearrangements. The chemical nature of the main substance used can now be stated. It is dichloro-diethyl-sulphide, or mustard gas. Three other substances of similar efficiency were found, all of them chemically related to mustard gas. Lewisite, on the other hand, gave negative results. The results were first described in a report sent to the Ministry of Supply on March 14, 1942.

In a large-scale test on the production of mutations by mustard gas carried out in April 1941, *Drosophila melanogaster* males were exposed to volatilized mustard gas and afterwards tested for sex-linked lethals by the standard CIB method. 7·3 per cent lethals were obtained in more than 1,000 chromosomes, as compared with 0·2 per cent in the controls. In later tests, even higher mutation-rates (up to about 24 per cent) were obtained. The limit for the increase in mutation-rate by increase of dosage is given by the equally increased rate of dominant lethality in the $F_1$: doses which produce more than 20 per cent sex-linked lethals practically sterilize all the treated males.

The mutagenic action of mustard gas appears to be exercised directly on the chromosomes, and not by way of a change occurring primarily in the cytoplasm; for the mutation-rate is not increased in untreated spermatozoa which have been introduced into treated eggs. Various rearrangements (inversions, large deletions, translocations) have been produced by the treatment. But the frequency of translocations is lower than would be expected after a dose of X-radiation which produces the same percentage of sex-linked lethals; it is, however, still considerably higher than after ultra-violet treatment. Thus, in one experiment with mustard gas in which the frequency of sex-linked lethals was 8·6 per cent, only seven translocations involving the *X*-chromosome and/or the two large autosomes were found in 812 treated chromosome sets.

The visible mutations which have so far been produced by chemical treatment have, with one exception, been previously described. In the progeny of chemically treated males, 30–50 per cent of visible mutations occur as 'fractionals', a proportion which is considerably higher than that observed in progenies of X-rayed males.

Full details of these results will be published elsewhere.

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Nov. 19.

[1] Auerbach and Robson, *Nature*, 154, 81 (1944).

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## Action of Mustard Gas on the Bone Marrow

IN their article on "Biochemical Research on Chemical Warfare Agents", Dixon and Needham[1] refer to the work of Wormall and his co-workers[2] on the distribution of mustard gas (H.) in the organs of rabbits which have been injected with a preparation of H. containing radioactive sulphur. It was found that the bone marrow contained only about one twentieth of the amount detected in the kidneys and lungs. Dixon and Needham go on to say: "It is surprising that marrow, the tissue most damaged, had the lowest H. content, while the two tissues with by far the highest H. content are practically undamaged by H. poisoning". They then develop a theory to account for these findings.

This interpretation, however, ignores the finding that mustard gas exercises drastic effects on the nucleus. It is capable of breaking chromosomes and thus interferes with mitosis or inhibits it altogether[2–4]. This effect will obviously be observed essentially in still actively dividing tissues, which will thus be expected to be particularly sensitive to the action of mustard gas. In accordance with this expectation, it has been found[5] that in the adult *Drosophila*, in which the only organ with actively dividing cells is the gonad, mustard gas produces a selective action on gametogenesis, while in developmental stages of the same flies, doses of mustard gas which affect the germ cells are usually harmful or definitely lethal to the animal as a whole, presumably because cells in many other tissues are also actively dividing.

In adult mammals the bone marrow is one of the few tissues in which cell division is actively proceeding. It is, therefore, not surprising that it is also highly sensitive to the action of mustard gas, even though it only contains comparatively small amounts of it.

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[5] Auerbach, C., and Robson, J. M., *Proc. Roy. Soc. Edin.*, in the press.

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# .—The Production of Mutations by Chemical Substances.

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(MS. received March 2, 1946. Read June 3, 1946)

## 1. Introduction

'HE production of mutations by the action of chemical substances on germ cells has often een reported. However, the variability of the spontaneous mutation rate and its dependence ot only on environmental conditions and physiological factors, but also on the genotype, iake it extremely difficult to assess the value of tests in which only small increases over the ontaneous mutation rate have been found. For this reason, Muller was still able to conclude 1941 that there was no definite proof that chemical substances could exert an effect on e mutation rate. Since then, Thomas and Chevais (1943) have reported results with lphonamides which, if they can be confirmed, would indicate a real, though slight, action these substances on the chromosomes, at least as far as gene mutations are concerned. ubbe (1940), working on plant material, observed a significant increase in mutation rate th phenol and potassium thiocyanate. It is of interest that Auerbach and Robson (1943) dependently observed a similar effect with allyl isothiocyanate in experiments on *Drosophila*. ven these definite effects are, however, very slight.

During the last four years we have been testing a number of chemical substances. Among ese a certain group has been found which increases the rate of occurrence of mutations d chromosome rearrangements to a similar extent as that brought about by X-rays and nilar physical agencies. The best known representative of this group is mustard gas, d the present report deals only with the effects produced by this substance. Results tained with other effective substances will be published later.

The use of mustard gas as a mutagenic substance was suggested by the observations that the substance produces a prolonged inhibition in the mitotic activity of the vaginal thelium of the mouse without any appreciable histological effect, *i.e.* that mustard gas is able of producing a prolonged effect on nuclear activity; and (2) that there are resemblances ween burns produced by X-rays and by mustard gas. Both types of lesion are slow in aling and show a tendency to break down again after apparent healing. Since X-rays known to act on the chromosomes, it was thought possible that mustard gas might have imilar action.

## 2. Material and Methods

*Drosophila melanogaster* was used in all experiments.

In the first experiments on the effect of mustard gas, the flies were exposed to the gas in a ge glass chamber (capacity 1 litre); fixed amounts of liquid mustard gas were volatilized heating in the presence of the flies. It was found that, using this method, the results were iable.

Fig. 1 illustrates the method finally adopted. The flies were contained in a glass tube × 1″), enclosed at both ends with porcelain filter discs. One end of the chamber was nected through a T-tube to an atomizer spray containing a solution of mustard gas in lohexane, and to a constant air flow (2 litres of air per minute). The mustard gas was ayed at regular intervals for fixed periods of time and was swept through the exposure mber by the stream of air. In a typical experiment a 1 : 10 mixture of mustard gas and lohexane was sprayed at 10-second intervals for periods up to 15 minutes. The amount nustard gas thus administered could be varied by altering either the concentration of the ution, the interval between sprays, or the period of spraying.

The concentration of mustard gas produced in the exposure chamber by the direct method cribed above was too high for *Drosophila* eggs and larvæ, for which the indirect method

illustrated in fig. 2 was used. The eggs were placed in the exposure chamber lying on p of agar food on a glass slide, while the larvæ were contained in a small glass tube (3" ( = 7 pe enclosed at both ends with fine bolting silk. The chamber was connected to a re cultures in addit

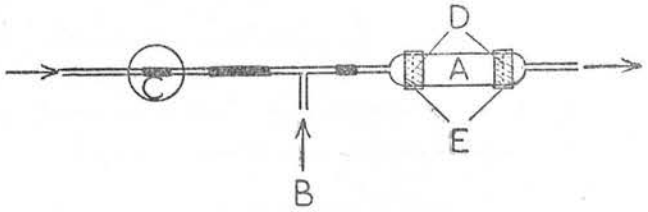


FIG. 1.—Showing the apparatus used for exposing the flies to mustard gas.
A = Exposure chamber. C = Flowmeter.
B = Spray entry. D = Porcelain perforated discs.
E = Rubber tubing.

aspirator bottle into which the mustard gas solutions were sprayed at constant interva sterilizi fixed periods, and where the mustard gas spray was mixed, diluted, and swept in inspecti exposure chamber by a constant air flow of 2 litres per minute. After the sprayin insemin

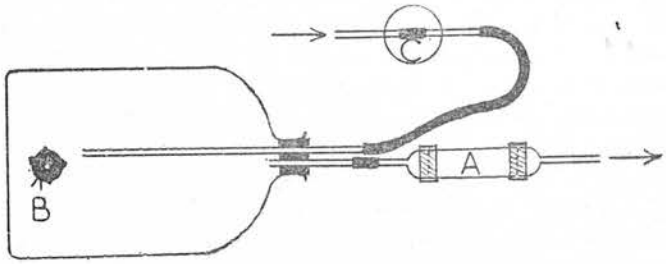


FIG. 2.—Showing the apparatus used for exposing eggs and larvæ to mustard gas.
A, B, and C as in fig. 1.

finished, the mustard gas accumulated in the mixing and diluting chamber was swept t the exposure chamber for a further 90 seconds before it was disconnected. In these ments with both the direct and indirect methods the temperature was not controlled.

Certain difficulties which are inherent in the use of biological material will be di in the appropriate sections.

Results.

## 3. The Production of Recessive Lethals

The first test for the production of sex-linked lethals by mustard gas was carried April 1941. ♂♂ of wild-type stock (Oregon-K) were exposed and then subjected to t test. The result left no doubt as to the potency of mustard gas as a mutation-pr agency. Later tests, carried out in connection with other experiments, brought confirmation. In Table I the data for three different experiments are summarized.

TABLE I.—The Frequency of Sex-linked Lethals after Treatment of ♂♂ with Mustard Gas (*CIB* Method)

| Serial No. of Experiment | Strain | No. of Tested X-Chromosomes | X-Chromosomes in which a Lethal developed | | No. of X-Chromosomes in which a Semi-Lethal developed | Le Semi- |
|---|---|---|---|---|---|---|
| | | | No. | Per cent. | | Per |
| H13 | Oregon-K | 1231 | 90 | 7·3 | 11 | |
| H32 | ,, | 790 | 68 | 8·6 | 4 | |
| H60 | *dp*; *e* | 115 | 28 | 24·2 | 0 | |
| Control for H13 | Oregon-K | 1216 | 3 | 0·25 | 0 | |

In the $F_1$ of the treated series in the first experiment there were 94 sterile cultures (= 7 per cent.). The difference from the controls, which contained 44 (= 3·5 per cent.) sterile cultures, is statistically significant, and suggests the induction of dominant sterility mutations in addition to recessive sex-linked lethals.

Although no special attempt was made to detect visible mutations in these first tests, a number of them were found incidentally. Some of them occurred among the semi-lethals, others had normal viability. A few of the lethals were found to be associated with chromosome rearrangements which were large enough to be detected by rough location tests. A cytological analysis of about 100 lethals obtained in the first experiments has been carried out by Slizynski and Slizynska (1946).

### *The Production of Dominant Lethals*

Hatchability of eggs laid by treated ♀♀, or by untreated ♀♀ mated to treated ♂♂, is reduced to a degree which depends on the dose. Even with high doses which have a complete sterilizing effect, willingness to copulate persists, and the sperm remains motile as seen by inspection of the seminal receptacles of ♀♀ which have been treated themselves or have been inseminated by treated ♂♂. The possibility has to be considered that treatment either of the sperm or of the ova might create conditions which make fertilization impossible, even though motile spermatozoa are present in the female genital tract. This could only be decided by cytological examination of a sample of eggs, such as has been carried out for X-rays by Sonnenblick (1940), and Demerec and Kaufmann (1941). However, the fact that with medium doses a certain percentage of eggs develop into larvæ shows that a proportion, at least, of the ova become fertilized. Moreover, the ability of mustard gas to break the chromosomes, which had been indicated by the first experiments and subsequently confirmed, makes it probable *a priori* that some of the induced chromosome rearrangements will be of the kind which cause death of the zygote. It seems therefore likely that the reduction in hatchability after treatment of either father or mother with mustard gas is at least partly due to dominant lethality, which has also been shown to be responsible for the low hatchability observed in X-ray experiments on *Drosophila* (Sonnenblick, 1940; Demerec and Kaufmann, 1941).

### *Inhibition of Gametogenesis*

Impaired hatchability of eggs is not the only reason for the reduced fertility of treated flies. Both oogenesis and spermatogenesis are interrupted by the treatment as shown by the following observations:—

(*a*) *Oogenesis.*—When ♀♀ are exposed to sub-lethal doses of mustard gas and subsequently kept on food with ♂♂, the number of eggs produced varies greatly in different individuals. This variation was found to be due, to some extent at least, to the different stages of development at which the ovaries were exposed. In virgins which are treated soon after hatching, or which are kept on a starvation diet from the period of hatching to that of exposure, the ovaries remain tiny and contain mostly young and degenerating follicles without properly formed walls. When such ♀♀ grow older, their abdomen expands and becomes filled with fatty tissue. Sometimes a few eggs are laid, and then the basal portion of the ovariole which produced the egg remains an empty sac. On the other hand, ♀♀ whose ovaries at the time of treatment are already fully developed may lay fairly well for a number of days. The most striking feature about the ovaries of such ♀♀ is the discontinuity of the developmental stages within the egg strings. Eggs ready to be laid are often in immediate proximity to quite small and undeveloped follicles, the intermediate stages being missing. Towards the end of the fertile period this peculiarity becomes very pronounced. Finally, the ovaries become very thin, with empty basal portions and degenerating young follicles in the apical parts. These observations show that only ova which have, at the time of treatment, passed a certain critical stage are able to reach maturity after exposure of the ♀♀ to mustard gas.

(*b*) *Spermatogenesis.*—No histological study was made of the effects of mustard gas on the testes. Since, however, ♂♂ treated even with high doses do not lose their willingness to copulate, the length of the period following treatment during which they retain their capacity

of inseminating ♀♀ will give a rough indication of the stage at which spermatogenesis becon interrupted. The following experiment was carried out to determine this period:—

A sample of some fifty wild-type ♂♂ was divided into 4 groups, namely:

*VT* kept without ♀♀ up to the time of exposure.

*VC* controls for VT.

*MT* mated *en masse* to about twice their own number of virgin ♀♀ on the day preced exposure.

*MC* controls for MT.

After exposure of the T-groups all ♂♂ were mated individually to two virgins each. Ev 2–3 days these ♀♀ were exchanged for fresh ones. On the 13th day following treatment, last batch of ♀♀, which had been put with the ♂♂ on the 10th day, was dissected, and seminal receptacles were examined for the presence of spermatozoa. The result is shown Table II.

TABLE II.—INSEMINATING CAPACITY OF ♂♂ TREATED WITH MUSTARD GAS ON THE 10TH AND 13TH DAY FOLLOWING EXPOSURE

| Series | No. of ♂♂ | No. of ♂♂ which, out of two ♀♀, had inseminated: | | |
|---|---|---|---|---|
| | | Both | One | Neither |
| VC | 10 | 10 | .. | .. |
| MC | 9 | 9 | .. | .. |
| VT | 10 | 1 | 4 | 5 |
| MT | 17 | 3 | 2 | 12 |

VT = virgin ♂♂.
MT = previously mated ♂♂.
VC and MC = controls to VT and MT.

Thus 10 days after exposure, insemination of at least one ♀ was still possible for hal the ♂♂ which, at the beginning of the experiment, had a full supply of sperm, but for less t one-third of those whose store of sperm had been depleted by previous matings. Dissec of a few of the treated ♂♂ showed that the testes were almost empty of spermatozoa. T test was repeated 23 days after exposure on some of the ♂♂. Sperm was found in 10 out of 1 in the control series, but only in one out of 15 in the treated series. If this one exception not due to some experimental error (*e.g.* non-virginity of the ♀) it may be a case of recuperat From these data it appears that the critical stage for inhibition of spermatogenesis by must gas coincides with the stage at which, after X-ray treatment, a drop occurs in the freque of induced recessive lethals (Harris, 1929; Hanson and Heys, 1929). Demerec and Kaufm (1941) observed a drop in X-ray induced dominant lethality as late as 19 days after treatm but since they did not keep the ♂♂ continuously with the ♀♀, their data are not quite compara to ours.

### *Difference in Sensitivity to Mustard Gas*

Very early in the course of the work it was found that one of the main difficulties con in finding a method of standardizing the dose so that the results of different tests are compar with one another and with results of similar tests carried out with X-rays. Unfortuna the apparatus used in these experiments did not allow control of temperature and humi during exposure. But even where it is possible to administer the same amount of gas u strictly comparable conditions, the actual amount which can penetrate to the chromoso under test—whether they be in the germ cells or in somatic cells—depends on anatomical physiological factors which vary not only in different lines and individuals of the same but also at different stages of development. Thus the same dose, as measured by phys

constants, corresponds to quite different effective doses when administered to eggs, larvæ, or imagines; to ♂♂ or ♀♀; to large or small flies, etc. Bodily activity during exposure is also of some importance. This is illustrated by the results of two tests in which the ♂♂ to be exposed were divided into two groups, one group being deprived of their wings previous to exposure. Altogether 807 eggs were tested for hatchability. Of 361 eggs fertilized by wingless ♂♂ 137 hatched, while of 446 eggs fertilized by winged ♂♂ only 29 hatched.

The simplest way of assessing the genetical effectiveness (*i.e.* the amount of gas reaching the chromosomes) of doses applied to adult ♂♂ consists in measuring the degree of dominant lethality induced in their progeny. Using this criterion, it was found that susceptibility is but slightly influenced by age and previous rearing conditions, unless the latter are so extreme that they result in marked reduction of body size with a consequent relative increase in body surface. The most important source of variation in sensitivity to a given dose is undoubtedly the existence of genetical differences between different strains and different individuals. When ♂♂ of various strains are exposed together and in the same container, the percentages of dominant lethality in their progeny show marked and consistent differences, a certain range of variation being characteristic for a given strain. By using tested strains as standards of comparison for new stocks to be tested, it was possible to grade the various strains according to their sensitivity; the least susceptible stock gave a hatchability of 70 per cent. with a dose which completely sterilized the most susceptible one (Table III).

TABLE III.—VARIATION IN SUSCEPTIBILITY TO MUSTARD GAS IN DIFFERENT STRAINS, AS MEASURED BY THE PERCENTAGE OF DOMINANT LETHALITY AMONG THE PROGENY OF TREATED ♂♂

| Strain | Dose | No. of Independent Tests | No. of ♂♂ in all Tests | No. of Eggs Laid | Percentage of Eggs Hatched | Percentage Hatched in Separate Tests |
|---|---|---|---|---|---|---|
| Florida-4 . . | I | 3 | 49 | 916 | 73·2 | 70·0<br>70·8<br>80·7 |
| *sc*$^{81}$ *In S w*$^{a}$ *sc*$^{8}$ . | I | I | 5 | 96 | 54·2 | .. |
| ,, . . | II | I | 3 | 60 | 63·3 | .. |
| *w sn*$^{3}$ *B* . . | I | I | 10 | 153 | 31·4 | .. |
| ,, . . | II | I | 4 | 80 | 62·5 | .. |
| Oregon-K . . | I | 3 | 32 | 805 | 12·5 | 6·9<br>10·9<br>17·3 |
| *B. Y*$^{S}$*/sc. Y*$^{L}$ . | I | I | 10 | 364 | 0·0 | All 0·0 |
| *f. Y*$^{S}$*/sc. Y*$^{L}$ . . | I | 3 | 32 | 613 | 0·0 | |
| ,, . . | II | I | 8 | 160 | 0·0 | |

*Note.*—Dose II, as measured by physical constants, was about 25 per cent. lower than Dose I.

Although variability is high both between individuals used in the same test and between repeated tests carried out with the same dose on the same stock, the data clearly show genetical differences in sensitivity. Thus of the two wild-type stocks, Florida-4 has a much higher tolerance than Oregon-K. The sensitivity of the two stocks carrying the *sc. Y*$^{L}$ translocation is so exceptionally high that it seemed to suggest an influence of this chromosomal rearrangement on the susceptibility of the spermatozoa themselves. To test this possibility, *f. Y*$^{S}$*/sc. Y*$^{L}$ ♂♂ and Florida-4 ♂♂ were mated to ♀♀ of the same unrelated line (*XX*) and the inseminated ♀♀ were then exposed together to mustard gas. Virgin ♀♀ of the same line were exposed simultaneously and mated subsequently to untreated brothers in order to supply a correction for the effect of the treatment on the maternal chromosome set. The result is shown in Table IV. The fact that the striking difference in dominant lethality among the progeny of treated ♂♂ of the two strains disappeared completely when the spermatozoa were exposed in identical environment, shows that this difference is determined not by the chromosomal constitution of the spermatozoa themselves, but by genetically determined differences

in the anatomy and the physiology of flies of the two stocks. ([redacted] nlikely [redacted] he treat [redacted] he treat [redacted] nuch ca [redacted] etween [redacted] The [redacted] ex-linke [redacted] etween [redacted] ccount [redacted] vithin th [redacted] The [redacted] genes fo [redacted] Among [redacted] o occur [redacted] ne exce [redacted] igh deg [redacted] nutation [redacted] ccurred [redacted] nustard [redacted] owever, [redacted] amples [redacted] nutation [redacted] ot to a [redacted] nstable [redacted] The [redacted] reatmen [redacted] ublicati [redacted] As re [redacted] H13, T [redacted] pecial t [redacted] ut in L [redacted] ottles t [redacted] ethals (s [redacted] back-cro [redacted] n $F_2$ of [redacted] all flies [redacted] therizat [redacted] $\delta\delta$ in [redacted] esult lef [redacted] ime it in [redacted] inked le [redacted] This con [redacted] echniqu [redacted] lone (E [redacted] H60). [redacted] In th [redacted] xpected [redacted] f musta [redacted] 1940, T [redacted] is the 3/ [redacted] omes II [redacted] ve also [redacted] s).

TABLE IV.—SUSCEPTIBILITY OF SPERM OF TWO DIFFERENT STRAINS TREATED IN THE BODY OF ♀♀ OF THE SAME UNRELATED STRAIN ($y$)

| Type of Sperm | No. of Treated ♀♀ | No. of Eggs | Percentage Hatchability * |
|---|---|---|---|
| Untreated . . . | 8 | 251 | 41 |
| Florida-4, treated . . | 7 | 206 | 7 |
| *f*. $Y^S/sc$. $Y^L$, treated . | 11 | 347 | 7 |

* Since attached-X ♀♀ were used for the test, the maximum possible hatchability of eggs from untreate was 50 per cent.

In view of the wide variation in the actual dose necessary to produce a genetical effe seemed desirable to use a biological method for assessing the effectiveness of a given dos the chromosome material. The *CIB* test was chosen for this purpose and was always ca out as a routine on an aliquot of the treated flies. This method makes it possible not on compare quantitatively the results of different experiments carried out with mustard gas also allows of a comparison with X-ray experiments. In the latter case, the dose in a g mustard-gas test is taken to be equivalent to an X-ray dose in *r*-units which produces the percentage of sex-linked lethals. We are well aware of the limitation of such a compar which is based on merely one of the effects which follow an action of these agents on nucleus. At present, however, this seems the only method available; but its limitations to be borne in mind in discussions based on such a comparison.

### *The Production of Visible Mutations*

It has already been mentioned that in the tests for lethals some visible mutations obtained. In order to detect any specific effects which mustard gas might exert, a sp experiment was carried out. Treated wild-type (Oregon-K) ♂♂ were mated to attached-X and their sons were examined for visible abnormalities. An equivalent number of sons untreated Oregon-K ♂♂ and attached-X ♀♀ was examined in order to determine what here

TABLE V.—THE PRODUCTION OF VISIBLE MUTATIONS BY MUSTARD GAS (TREATED OREGO ♂♂ MATED TO ATTACHED-X ♀♀)

| Group | Total No. of ♂♂ Examined | Total No. of Aberrations | Genetic Aberrations | | Non-gen Aberrati |
|---|---|---|---|---|---|
| | | | Proved by Breeding Test † | Presumed by Phenotype | |
| Treated . . . | 2750 | 148 | 51 | 28 | 69 |
| Control . . . | 2750 | 34 | 4 | 2 | 28 |

* A breeding test was performed only on a fraction of the ♂♂ listed in the last column. The remainde judged to be non-genetic because of their phenotype (see Appendix).

† Of these, 10 were sex-linked recessives and 41 autosomal dominants in the treated group. All t the control group were autosomal dominants.

and non-hereditary aberrations occur normally in these stocks. A small *CIB* test carri on some of the ♂♂ yielded 3 lethals in 68 chromosomes, enough to show that the dose ha effective. Breeding tests to determine the nature of the aberrations were successfully out on about 50 per cent. of the aberrant ♂♂ in both control and treated groups. In T the results are summarized; details are given in the Appendix.

Among 2750 sons of treated ♂♂ there occurred 79 mutations or presumed mutati compared with 6 among the same number of sons of untreated ♂♂. Since, however, i

nlikely that the frequency of non-inherited modifications should be significantly greater in he treated than in the control group, it is quite possible that a number of the abnormalities in he treated group classified by phenotype as non-genetic were, in reality, genetic, and that too nuch caution was exercised in this classification. If this is true, it would make the difference etween treated and control group even larger.

The proportion of sex-linked visible mutations was 11 in 2750 = 0·4 per cent., that of ex-linked lethals (in the *CIB* test) was 3 in 68 = 4·4 per cent. This gives a ratio of 1 : 11 etween the two frequencies. Although the error attached to this value is considerable on ccount of the small size of the *CIB* sample, it is clear that the real value lies somewhere ithin the range observed for irradiated material (*cf.* Muller, 1941).

The data do not provide evidence that mustard gas singles out specific genes or groups of enes for its action. Autosomal *Minutes* formed the majority of the inherited abnormalities. Among the sex-linked recessives, *rough eyes*, *rudimentary wings*, and *forked* were the only ones o occur more than once. In addition, there were two somatic mutations to *lozenge*. With ne exception all the mutations observed more than once affected loci, which also show a fairly igh degree of mutability after treatment with X-rays. The exception was a new sex-linked nutation "bashed" affecting the shape of the eye (Auerbach *D.I.S.*, XVIII, and XIX,), which ccurred three times after treatment with mustard gas and once after a combined treatment with nustard gas and X-rays, in all four cases in the inbred Florida-4 stock. It is not possible, owever, to assume that the same mutation cannot be produced by X-rays, since only small amples of this stock have been tested with X-rays for the occurrence of visible sex-linked nutations. The repeated occurrence of *bashed* in these experiments may therefore be due, ot to a specific action of mustard gas, but to the presence in the Florida-4 stock of a relatively nstable normal allelomorph at this locus.

The most striking feature in all experiments on visible mutations after mustard-gas reatment was the high percentage of mosaics. This will be dealt with in a separate ublication (Auerbach, ~~in the press~~).

## *The Production of Translocations*

As reported ~~in Section 3~~, a few of the sex-linked lethals produced in the first experiment H13, Table I) were found to be associated with large chromosome rearrangements. A pecial test to determine whether mustard gas actually produces translocations was carried ut in December 1941. Treated wild-type (Oregon-K) ♂♂ were mated in the same culture ottles to *CIB*/*scar* and to *y*; *bw*; *e* ♀♀. Their Bar daughters were used in a test for sex-linked ethals (see Experiment H32, Table I). Their phenotypically wild-type sons $\left(\frac{dp}{+}; \frac{e}{+}\right)$ were ack-crossed individually to virgins of the *y*; *bw*; *e* stock. This method allows of the detection n $F_2$ of translocations between X and II (no *dp* sons), X and III (no *e* sons), and II and III all flies either *dp e* or wild-type). Since inspection of the cultures was carried out without therization, only those translocations were detected in which the aneuploid classes (*e.g.* the *dp* ♂♂ in an X-II translocation) were completely or almost completely non-viable. The esult left no doubt about the ability of mustard gas to produce translocations, but at the same ime it indicated that, compared with a dose of X-rays producing the same percentage of sex-inked lethals, mustard gas is considerably less effective in the production of translocations. This conclusion was confirmed in two subsequent experiments in which similar genetical echniques were used for detecting the presence of translocations in the two long autosomes lone (Experiment H89), or in the Y-chromosome and the two long autosomes (Experiments H60). The data from all three experiments are collected in Table VI.

In this table, the last column represents the percentage of translocations which would be xpected with a dose of X-rays producing the same frequency of sex-linked lethals as the dose f mustard gas used. The expected frequencies have been calculated from the data of Muller 1940, Tables II and V), and using Muller's rule that the frequency of translocations varies s the 3/2th power of the dose. As Muller's data refer only to translocations between auto-omes II and III they are only directly comparable with Experiment H89. In Experiment H32 ve also recorded exchanges involving the X-chromosome, and this increases the expected

number of translocations by at least one-third (Muller and Altenburg, 1930; Patterson, Stone, Bedichek and Suche, 1934). In Experiment H60 the heterochromatic Y-chromosome was included in the test instead of the X-chromosome. Since, as Neuhaus (1939) has shown,

TABLE VI.—FREQUENCY OF TRANSLOCATIONS PRODUCED BY MUSTARD GAS

| Experiment | Percentage Sex-linked Lethals * | No. of Fertilized Back-cross Cultures | Chromosomes Tested for Translocations | Translocations | | |
|---|---|---|---|---|---|---|
| | | | | No. of Tested Chromosome Sets | Per cent. | Expected Percentage (approx.) † |
| H32 | 8·6 | 816 | X, II, III | 7 ‡ | 0·9 | 7 |
| H89 | 14·5 | 981 | II, III | 21 | 2·1 | 10 |
| H60 | 24·2 | 33 | Y, II, III | 1 | 3·0 | More than 10 |

* In a parallel test on ♂♂ of the same treated batch.
† With a dose of X-rays giving the same percentage of lethals.
‡ 4 II–III, 1 I–II, 2 I–III, 1 I–II–III.

♂♂ carrying a translocation of the Y-chromosome are usually sterile, this entailed the use in $F_1$ of ♀♀ instead of ♂♂, and the introduction of large inversions to prevent crossing-over. In detail the scheme was as follows:—

$P_1$ ♂♂ *dp*; *e* treated × ♀♀ $\underline{y}$; $\frac{Cy}{Bl\ L^2}$; $\frac{D}{LVM}$ mass.

$F_1$ ♀ $\underline{y}$; $\frac{Cy}{dp}$; $\frac{LVM}{e}$ (phenotype *Cy*, non-*D*) × ♂ *dp*; *e* pairs.

$F_2$ The segregation for sex, *dp* and *e* was used to determine whether a translocation was present.

Unfortunately, sterility in this experiment was so high that only 33 chromosome sets could be tested; but, since only one translocation occurred with a dose producing 24 per cent. lethals, the result is in agreement with the other two experiments.

### *The Production of Large Deletions*

In two experiments, treated ♂♂ were mated to $\underline{y\ v\ f}$ ♀♀, and their daughters were examined for the presence of hyperploid ♀♀ carrying a paternal X-chromosome with a large deletion. These ♀♀ are recognizable by the fact that one or two of the three marker genes are covered

TABLE VII.—FREQUENCY OF LARGE DELETIONS IN THE PROGENY OF TREATED WILD-TYPE ♂♂ AND $\underline{y\ v\ f}$ ♀♀

| Experiment | Mutagenic Agent | Percentage Sex-linked Lethals * | No. of $F_1$ ♀♀ Examined | Large Deletions | | |
|---|---|---|---|---|---|---|
| | | | | Observed No. | Expected No.* | Difference |
| H92 | Mustard gas | 9 | 6635 | 16 | 41 | 25 ± 6·4 |
| HX1 | ,, | 6 | 5052 | 9 | 18 | 9 ± 4·2 |
| HX1 | X-rays, 2000 *r* | 7 | 4368 | 14 | 15 | .. |

* In a parallel test on males of the same treated batch.
† With a dose of X-rays giving the same percentage of lethals.

up by the presence of their normal allelomorphs in the deleted fragment. The data, which are summarized in Table VII, prove that mustard gas can induce large deletions in treated sperm.

The "expected number of deletions" shown in Table VII has been calculated on the assumptions (1) that an X-ray dose of 4000 *r* produces about 1 per cent. deletions of the types detectable in our experiments (Bishop, 1938; Pontecorvo, 1940), and (2) that the frequency of deletions varies with the 3/2 power of the dose (Muller, 1940). The result of the X-ray experiment carried out by us (last row of Table VII) confirms that these assumptions are justifiable.

In both experiments with mustard gas the observed number of deletions was significantly smaller than expected, but the divergence was not as marked as in the case of translocations.

### *Effect of Treated Cytoplasm on Untreated Chromosomes*

The possibility has to be considered that mustard gas exercises its effect not directly on the chromosomes, but indirectly by a chain of reactions which starts in the cytoplasm and finishes in the chromosomes. If this were so, then one would expect mutations to develop in untreated chromosomes which have been introduced into treated cytoplasm and have repeatedly divided

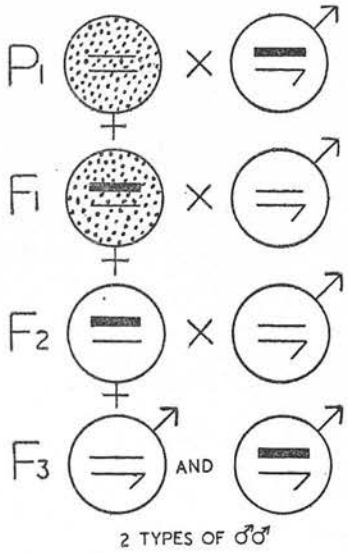


FIG. 3.—Scheme for detecting the possible mutagenic action of cytoplasm treated with mustard gas on an untreated chromosome introduced into it.

Stippling indicates treated cytoplasm. = X-chromosome under test.

Absence in the $F_3$ of ♂♂ with the tested chromosome indicates that a lethal has been produced in this chromosome during its sojourn in the treated cytoplasm of the $F_1$.

in this environment. Such conditions can be created by mating untreated ♂♂ with treated ♀♀ and recording the frequency of lethals which develop in the paternal chromosomes. A positive result, *i.e.* an increased frequency of lethals in the tested chromosomes as compared with similar chromosomes introduced into untreated ♀♀, would indicate either that mustard gas persists in the cytoplasm for a sufficiently long time, and in a sufficiently high concentration, to affect the chromosomes during cleavage and gonad formation, or that a mutagenic substance is formed in the treated cytoplasm. A negative result, on the other hand, would mean that no mutagenic dose of either mustard gas or of a reaction product of mustard gas with the cytoplasm was present during development.

The method was a modification of that used by Muller (1930) and Timoféeff-Ressovsky (1937) for analysing the mutagenic action of X-rays. The essential features of our method are represented in fig. 3, in which the treated cytoplasm is indicated by stippling, and the untreated chromosome under test by a thick line. The ova from which the $F_1$ developed were treated in the body of the $P_1$ ♀♀. A few hours after treatment the $P_1$ ♀♀ were mated to the untreated $P_1$ ♂♂. A lethal which arises in the paternal X-chromosome during embryonic development of an $F_1$ ♀ will result in a ♀ which carries the new lethal in part of her body and presumably in most cases only in part of her gonads. A proportion of her daughters, therefore,

will be heterozygous for the lethal and will have no sons with the tested chromosome in $F_3$. Hence a mutagenic action of the treated cytoplasm is detected by an increased mutation rate in the $F_3$ as compared with controls.

The main difference between our method and that used by the above-mentioned workers consists in the use of ♀♀ instead of ♂♂ in $F_2$. Since a ♀ is protected from the harmful effects of a recessive lethal on one X-chromosome through the presence in the other of the normal allelomorph, this method safeguards against the possibility that a lethal which arises early during development of an $F_1$ individual may either kill their carrier or else prevent the germ cells carrying it from developing into mature gametes. The use of ♀♀ in $F_1$ entailed the introduction of inversions to prevent crossing-over. In detail the scheme was as follows:—

$P_1$ ♀♀ *w sn*$^3$ *B* (treated) × ♂♂ *sc*$^{s1}$ *In S w*$^a$*sc*$^8$ (untreated) mass.

$F_1$ ♀ $\frac{w\ sn^3\ B}{sc^{s1}\ In\ S\ w^a sc^8}$ (virgin) × ♂ *Or K* (wild-type) pairs.

The $F_2$ was examined for the presence of *w sn B* and *w*$^a$ ♂♂.

$F_2$ ♀ $\frac{Or\ K}{sc^{s1}\ In\ S\ w^a sc^8}$ phenotypically (non-Bar) × brother pairs.

The $F_3$ was examined for the presence of *w*$^a$ ♂♂.

To guard against the possibility that secondary non-disjunction in an $F_2$ ♀ mated to a *w*$^a$ brother might mask the presence of a lethal on the *w*$^a$ chromosome, the $F_2$ ♀♀ were collected from cultures which did not produce wild-type sons. An identical scheme of matings was carried through with controls.

The efficiency of the dose of mustard gas to which the cytoplasm was exposed was deduced from the incidence of lethals in the treated *maternal* X-chromosome. These lethals are detected by the absence of *w sn B* ♂♂ in $F_2$. Three lethals were found in 71 chromosomes from treated ♀♀, as compared with none in 76 controls. In addition, one of the 71 $F_1$ ♀♀ was found to be heterozygous for a lethal in the untreated *paternal* X-chromosome. If this lethal had not arisen spontaneously in the ♂, it may conceivably have been induced in the genital tract of the ♀ or in the ovum between copulation and the first cleavage division.

The results of the experiment are shown in Table VIII. It is clear that no mutagenic effect was produced in untreated chromosomes which underwent all the mitoses of embryogenesis in treated cytoplasm. It would appear therefore that, at least with the dose used,

TABLE VIII.—FREQUENCY OF SEX-LINKED LETHALS IN UNTREATED X-CHROMOSOMES WHICH HAVE BEEN INTRODUCED INTO TREATED CYTOPLASM

| Series | No. of Chromosomes Tested | No. of Lethals |
|---|---|---|
| Treated cytoplasm . . | 749 | 1 |
| Controls . . . . | 1234 | 1 |

the chromosomes react directly to mustard gas as they do to radiation, and not secondarily to modifications of the cytoplasm. It is of interest that the dose was high enough to produce 13 per cent. lethals in ♂♂ of the *sc*$^{s1}$ *In S w*$^a$ *sc*$^8$ stock which were exposed simultaneously with the $P_1$ ♀♀.

## DISCUSSION

The results reported here prove beyond doubt that mustard gas can produce effects on chromosomes and genes which are qualitatively and quantitatively comparable to those produced by X-rays and γ-rays. Similar results have been obtained with three other vesicants chemically related to mustard gas. These will be described in the future. It should be noted, however, that not all vesicants with a high power of penetration are necessarily mutagenic. A remarkable exception is lewisite. With a dose of lewisite which killed the majority of ♂♂

exposed to it, only 7 lethals were obtained in 1271 chromosomes from treated sperm, as compared with 8 lethals in 891 control chromosomes. It is, of course, possible that the dose of lewisite which is necessary to produce a mutagenic effect is so toxic that all the individuals which receive it die. If this were so, lewisite might still be shown to be mutagenic in an organism less susceptible to its toxic action.

Whether or not physical and chemical agencies exert the same primary actions on the chromosomes is one of the many problems which are opened up by these results. One of the attractions of chemical substances as mutagens lies in the possibility that there might exist affinities between specific chemical groups and certain parts of the genic material which, by the occurrence of selective effects, might provide evidence concerning the chemical structure of the chromosomes.

Specific reactions might occur at two levels. There might, in the first place, exist an affinity of the active substance for certain essential components of the chromosome, *e.g.* certain proteins or amino acids, nucleic acid or one of its constituents. Affinities of this kind would lead to the selective occurrence of certain types of effects, while other types with which this particular component is not concerned would not appear after treatment. If, for example, certain chemical substances could be shown to produce frequent mutations, but no small deficiencies, or *vice versa*, this would strongly suggest that different chemical processes are concerned in these two types of changes. In the second place, the specificity of the substance might be inferred if mutations of specific loci were selectively induced. It would suggest that the substance had a chemical affinity for these genes.

The present data give little, if any, indication of the existence of either type of specificity. Mustard gas and similar active substances have been shown to produce visible mutations, lethals, and gross rearrangements. Furthermore, Slizynski and Slizynska (~~in the press~~ 1946) have shown that small deficiencies are also produced. The visible mutations are comparable to those obtained by radiation both in the frequency of their occurrence and in their type. All the chromosomes, and—as location tests of the sex-linked lethals have shown (Slizynski and Slizynska, ~~in the press~~ 1946)—all euchromatic regions of at least one chromosome are susceptible. Two differences only have so far been found between the effects of X-rays and mustard gas, both of them quantitative, viz.:

(1) A dose of mustard gas produces fewer gross rearrangements (translocations and deletions) than a dose of X-rays which produces the same percentage of sex-linked lethals. This need not necessarily imply that mustard gas is less efficient than X-rays in the production of chromosome breaks. A gross rearrangement occurs when chromosomes are broken in one or more places and join up into a different arrangement. It is conceivable that the treatment with mustard gas favours restitution of broken chromosomes in preference to the formation of new reunions, especially if the breaks involve two different chromosomes. This would be in agreement with the findings that translocations, which, of course, involve two chromosomes, are relatively less frequent than the intrachromosomal deletions. This assumption would gain further support if it were found that the efficiency of mustard gas approached that of X-rays in the production of single breaks, and it is planned to test this.

(2) In the progeny of mustard gas-treated ♂♂, mosaics form a significantly higher percentage of all visible mutants than in the progeny of X-rayed ♂♂. This is dealt with in a separate publication (Auerbach, ~~in the press~~ 1946).

It is of interest to note that in respect of both these peculiarities, *i.e.* shortage of gross rearrangements and high percentage of visible mosaics in $F_1$, mustard gas appears to occupy a curiously intermediate position between X-rays on the one hand and ultra-violet radiation on the other. For it is less efficient than X-rays, and more efficient than ultra-violet rays, in the production of gross rearrangements, and, if Stadler's (1939) data on maize can be compared with our findings on *Drosophila*, the gas produces more mosaics than X-rays, but not quite as many as ultra-violet radiation.

In considering the essential similarity between the nuclear effects of mustard gas and related substances on the one hand, and those of X-rays and other ionizing radiations on the other hand, it has to be borne in mind that the same molecular reaction may be initiated both by physical and chemical stimuli. The ensuing changes may then be due merely to details of gene and chromosome structure which condition the response to the primary process. Such

considerations do not, of course, exclude the possibility that certain substances may be capable of producing specific effects; but they do suggest that large numbers of substances may have to be tried before one is found which possesses such specific effects.


## Summary

Data are presented which show that mustard gas is capable of producing lethals and visible mutations at rates which are comparable with the effects of X-rays. Up to 24 per cent. sex-linked lethals have been produced in experiments in which mature spermatozoa were treated. Other substances, related in chemical composition to mustard gas, have produced similar effects, and the results will be published later. Lewisite did not produce any such effects.

The induction of dominant lethals by these chemical substances, and their interference with gametogenesis, result in greatly reduced fertility of treated ♂♂ and ♀♀.

Appreciable differences in sensitivity to the gas have been observed in different individuals, different strains, and at different stages of development.

Deletions, inversions, and translocations are produced, but the proportion of translocations and, to a lesser degree, of deletions to sex-linked lethals is significantly smaller than after X-ray treatment.

The rate of lethal mutations was not increased in untreated chromosomes which had been introduced into cytoplasm treated with mustard gas. This suggests that mustard gas, like X-radiation, acts directly on the chromosomes.

The data obtained so far have given no evidence that mustard gas acts selectively on certain constituents, or at certain loci, of the chromosomes.


## APPENDIX

### Detailed Data of the Experiment on the Production of Visible Mutations ~~Reported in Section 7~~. (See Table V)

Of the 34 aberrants in the controls, 16 were not tested, or died before they had produced progeny. Most of these obviously were developmental modifications of the types always encountered when large numbers of flies are examined, such as blistered wings, crippled legs, abnormal abdomen; they also included one Minute ♂. Fourteen tested aberrants which did not transmit their abnormality to their progeny consisted mainly of the same type of modifications just mentioned. In addition, they included a fly whose right eye was bi-coloured for red and an eosin-like shade; the most likely interpretation of this case is the occurrence of a somatic mutation in one of the eye-forming cells. Three aberrations were inherited as autosomal dominants of low and irregular penetrance (tendency to notched wings, kinked bristles, rough eyes, irregular venation). Finally, there was one autosomal Minute. The Minute which left no progeny, and the somatic mutation to ~~lozenge~~ eosin were counted among the genetic changes.

Of the 148 aberrants in the treated series, 71 were not mated, or died before they could be mated, or did not leave progeny. Among them were 10 Minutes, in which the Minute character of the bristles was often accompanied by other abnormalities such as rough eyes, scalloped wings, irregular venation, which are usually associated with chromosome aberrations. In addition, there were 7 fractional Minutes, again most of them with additional signs of chromosomal unbalance in the affected part of the body. Six more ♂♂ with normal bristles showed similar indications of chromosomal unbalance either over their whole body surface or over part of it. One fly exhibited in one of its eyes what was obviously a somatic mutation to one of the members of the lozenge series. Thus of the 71 aberrants of which no progeny was obtained, ~~two~~ 27 were strongly suspected to be chromosome rearrangements or losses. They have been counted as genetic aberrations.

Of 25 aberrants which gave a negative result in a breeding test, one was obviously a somatic mutation to lozenge, two were fractional Minutes, and one had one very small eye of irregular outline, as in the eyeless mutant. These four flies, too, were included among the group of aberrations with a genetical basis.

The transmitted abnormalities consisted of 10 sex-linked recessives and 41 autosomal dominants. The sex-linked recessives included forked (twice), wavy, rudimentary (? twice). The other five were not subjected to location tests. They consisted of the following phenotypes: slender bristles; notched wings (semi-dominant); rough eyes (twice, one a semi-lethal); spread wings combined with abnormalities of venation (semi-lethal). In addition two ♂♂ which had been subjected to breeding tests on account of very slight phenotypical irregularities turned out to carry sex-linked lethals. Possibly these ♂♂ were gonosomic mosaics for a lethal, and the phenotypical abnormalities had been only incidental.

The autosomal dominants included 21 total and 13 fractional Minutes. Five abnormalities of bristles, wings, and eyes, all with low penetrance and irregular expression, were similar to those found in the control and were presumably carried in one of the stocks used in the experiment. Of the remaining two autosomal dominants, one was phenotypically like Delta and probably allelomorphic with it; the second, which first appeared as a fractional, was an allelomorph of $vg^D$ and phenotypically like $vg^D$, although cytologically probably not identical with it (Auerbach, *D.I.S.*, XVII).

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# Tests of chemical substances for mutagenic action

by

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The discovery in 1941 (Auerbach and Robson, first published in 1946) that mustard gas ($\beta$-$\beta'$ -dichloro-diethyl-sulphide), $(ClCH_2.CH_2)_2S$, is comparable to X-rays in its capacity to produce mutations and chromosome re-arrangements naturally raised the question as to what special properties of mustard gas enable it to act in this manner. There are indications (Auerbach and Robson 1946, in the press) that the mutagenic effects of mustard gas are due to a direct action on the chromosomes, and not to an indirect one via the cytoplasm. This suggests that a selective and specific chemical reaction occurs between mustard gas and the genic material. It appeared possible that a systematic survey of substances chemically related to mustard gas might reveal a chemical group or structural arrangement responsible for the mutagenic action. This, in its turn, might also throw some light on the other component of the reaction, the gene or chromosome, and on the process of mutation itself.

## Material and Methods

The substances to be tested were, with one exception, chosen on account of their chemical and pharmacological similarity to mustard gas. They were -

(1) N-methyl di-(2-chloroethyl) amine, $CH_3.N(CH_2.CH_2Cl)_2$, one of the so-called nitrogen mustards. This is a liquid with strong vesicant action. It decomposes fairly rapidly in water. Its hydrochloride is a solid, soluble in water and forming a stable solution. This solution proved useful in certain tests in which it was convenient to apply a mutagenic substance in aqueous solution.

(2) Tri-(2-chloroethyl )amine, $N(CH_2.CH_2Cl)_3$, another nitrogen mustard of high/-

high vesicant capacity.

(3) 2-2'-di($\beta$ -chloroethylthio)-diethyl ether, $O(CH_2.CH_2.S.CH_2.CH_2Cl)_2$. This is also a nitrogen mustard, but with rather weak vesicant action,

(4) Lewisite, $Cl_2$:As.CH:CH Cl, a potent vesicant gas.

(5) Mustard oil, allyl isothiocyanate, $CH_2$:CH.NCS. Pharmacologically this substance resembles mustard gas in that it has a slight vesicant effect. More striking, however, is its lachrymant action. It occurs naturally in a number of plant species of the genus Brassica. A preliminary communication on results obtained with mustard oil has already been published (Auerbach and Robson, 1944),

(6) Chloracetone, $ClCH_2.CO.CH_3$, and dichloracetone, $ClCH_2.CO.CH_2Cl$. These substances belong to the group of lachrymators and are thus pharmacologically related to mustard oil. Moreover, their chemical structure has a certain basic resemblance to those of the poison mustards and of mustard oil, as will be explained more fully in the discussion.

(7) Osmic acid and picric acid. Like mustard gas, these substances are fixatives of protoplasm with a high power of penetration,

(8) Ammonia. This was tested because of previously published data by Lobashov (1937), which appeared to indicate that ammonia has a slight mutagenic activity.

The nitrogen mustards, mustard oil, chloracetone, dichloracetone, and lewisite in the later stages of our work were applied as sprays, either pure or in solution in alcohol or cyclohexane. The method has been previously described in full (Auerbach and Robson, in the press). Its main feature consists in introducing the substance as a spray at regular intervals into the container with the flies, while simultaneously air is being sucked through the container at a constant rate (2 litres per minute in the present experiments, unless stated otherwise). In Table I, the spray interval is given in seconds, and the total time/-

time of exposure in minutes. A control test with cyclohexane alone showed that this had no effect when applied pure. No control test with alcohol alone was carried out; the fact that alcohol had been used as solvent in tests with negative result was taken as sufficient evidence of its non-mutagenic nature.

In the first experiment with lewisite the substance was vaporized in a large chamber, about 1 cubic meter. Ammonia and osmic acid were used as vapours to which the flies were exposed in small glass containers. Picric acid in aqueous solution was used for bathing dechorionated eggs.

In all experiments, with the exception of those carried out with nitrogen mustards, the dose was chosen high enough to kill a fair proportion of the exposed flies or embryos.

The flies used for the test were wild-type Drosophila melanogaster, belonging either to an inbred Florida-strain (Fo-4), or to a mass-cultured Oregon strain (OrK). All tests were carried out on treated ♂♂ with the one exception of the picric acid experiments in which the majority of the tested chromosomes were derived from ♀♀ which had been treated as embryos.

For the detection of induced mutations, the standard ClB test for sex-linked lethals was used throughout. Controls were taken repeatedly. Care was taken that the control ♂♂ came from the same culture bottles as the treated ♂♂. In table I, the control data are always put immediately behind the experiment for which they serve as check. They are only of limited value for comparisons with experiments carried out at a different time.

When a substance had given a positive result in the ClB test, it was subjected to a test for the production of chromosome breaks. Treated ♂♂ were mated to attached-X ♀♀ of the genotype y v f. If, as a result of chromosome breakage, a portion of a paternal treated chromosome is deleted, a daughter receiving the

fragment/-

Fragment will be of a hyperploid in which one or two of the marker genes may be masked by the presence of their normal allelomorphs in the fragmented paternal X. In order to determine whether the applied dose was mutagenically effective, a ClB test was always carried out on an aliquot part of the treated flies.

In many cases hatchability of eggs laid by untreated ♀♀, which had been inseminated by treated ♂♂, was compared with that of control eggs. Low hatchability was taken as an indication of dominant lethality, which in its turn presumably is due to chromosome breaks.

## Results

### 1. Sex-linked lethals

The results of the various ClB tests are summarized in Table I.

Table I/-

TABLE I.

Tests for the induction of sex-linked lethals by chemical substances (not including mustard gas). ClB method. Except where otherwise stated, exposure was done by the spray method, with an air supply of 2 litres per minute. The spray interval in seconds and the total time of exposure in minutes are given in column 5. The controls were always taken for the experiment which immediately procedes them in the table.

| Substance | | Experiment. | Stock | Dose | No. of treated chromosomes | No. of lethals | % of lethals | Mutagenic efficiency of substance. |
|---|---|---|---|---|---|---|---|---|
| Nitrogen | A (1) | I | OrK | 1:1 in cyclohexane, 20 sec., 7 min. | 69 | 9 | 13.0 | high, similar to X-rays, and mustard gas. |
| | | II | OrK | no record kept | 609 | 31 | 5.1 | |
| | | III | Fo4 | " " " | 629 | 34 | 5.4 | |
| Mustards | B (2) | | Fo4 | pure, 10 sec.; 5 mins. | 741 | 46 | 6.2 | |
| | | control | Fo4 | ——— | 1274 | 3 | 0.3 | |
| | C (3) | | Fo4 | pure, 10 sec., 5 mins. | 586 | 50 | 8.5 | |
| Mustard-oil, allyl-iso-thiocyanate (4) | | I | Fo4 | pure, 10 sec., 8 min. | 756 | 17 | 2.2 | low, but significantly positive. |
| | | II | Fo4 | pure, 10 sec., 9 min. | 878 | 19 | 2.2 | |
| | | control | Fo4 | ——— | 963 | 4 | 0.4 | |
| Chloracetone (5) | | I | Fo4 | 1:2 in abs.alc., 15 sec., 6 mins. | 957 | 10 | 1.0 | doubtful, probably slightly positive. |
| | | II | Fo4 | pure, 15 sec., 8 min. | 617 | 6 | 1.0 | |
| | | control | Fo4 | ——— | 864 | 1 | 0.1 | |
| | | III | Fo4 | pure, 15 sec., 9 mins. | 1448 | 8 | 0.6 | |
| | | IV | Fo4 | pure, 15 sec., 10 mins. | 184 | 6 | 3.3 | |
| | | V | Fo4 | pure, 15 sec., 11 mins. | 530 | 0 | 0.0 | |
| Dichloracetone (6) | | | Fo4 | pure, 15 sec., 8 mins. | 971 | 8 | 0.8 | |
| Ammonia | | | Fo4 | vapour, killing majority | 1082 | 5 | 0.5 | doubtful, probably negative. |
| Osmic acid | | | Fo4 | vapour, killing majority | 940 | 4 | 0.4 ( 2 visibles) | |
| Picric acid | | | Fo4 | bathing of dechorionated eggs | from ♂♂ :237<br>from ♀♀ :710 | 2<br>0 | 1.0<br>0.0 | |
| Lewisite (7) | | I | OrK | gas chamber, 18° C, 1.5 cc., 30 min. | 1271 | 7 | 0.6 | negative |
| | | II | OrK | 1:6 in abs.alc., 3 liter air, 60 sec., 6 min. | 774 | 2 | 0.3 | |
| | | control | OrK | ——— | 891 | 8 | 0.9 | |

(1) $CH_3.N(CH_2.CH_2.Cl)_2$ (2) $N(CH_2.CH_2.Cl)_3$ (3) $O(CH_2.CH_2.S.CH_2,CH_2.Cl)_2$

(4) $CH_2:CH.NCS$ (5) $Cl\ CH_2.CO.CH_3$ (6) $ClCH_2.CO.CH_2.Cl$. (7) $Cl_2:As.CH:CH\ Cl$.

It will be seen that from the point of view of mutagenic efficiency the tested substances arrange themselves into 3 main groups. The first group comprises the so-called nitrogen mustards. Their mutagenic action is established beyond doubt and is of an order of size comparable to that of X-rays or mustard gas. The second group so far contains only one substance, allyl isothiocyanate or mustard oil, which has a weak, but definitely positive action. The difference between the toll of mutations in Experiment II with mustard-oil and the controls is almost 5 times its standard error. The remaining group is formed of substances with doubtful or negative action on mutation rate. As a subgroup it contains chloracetone and dichloracetone which appear to exercise a slight mutagenic action. With the exception of Experiment V with chloracetone, mutation rates in all tests with these two substances were on the high side of the spontaneous mutability in our stocks or even exceeded it (Experiment IV). Moreover, in the one experiment which was done with simultaneous controls (II) the difference between the mutation rates in the treated and untreated samples is statistically significant ($P = 0.05$). A similar statistical significance attaches to the difference between the overall mutation rate in tests with the two chloracetones and the controls to Experiment II; but this kind of comparison is of doubtful validity for the estimation of mutagenic effects which do not go far beyond the normal range of variation in spontaneous mutability.

The data obtained with ammonia, osmic acid and picric acid do not exclude the possibility of a very slight mutagenic action of these substances, but they do not, on the other hand, make it likely. Very large scale experiments with careful controls would be required to decide this point.

The results with lewisite were definitely negative, in spite of the fact

that lewisite was by far the most toxic of all substances used. In Experiment II the majority of the exposed flies were dead on the day following treatment.

## II. Visible mutations

With one exception (see below), no special tests for the detection of visible mutations were carried out, but a number of these were found incidentally. They include Minute, lozenge, yellow, garnet, and bashed (see Auerbach 1944). One mutation to white eyes and one to a phenotype resembling rudimentary were found in less than 1000 cultures of the ClB test after treatment with osmic acid. This seemed to suggest that osmic acid, although incapable of producing appreciable numbers of lethals, might act selectively on certain loci. However, when ♂♂ which had been treated with a strong dose of osmic acid were mated to attached-X ♀♀, their male progeny of about 1700 sons contained no other aberrants but one Minute and one possible scute.

Of 33 Minutes which appeared after treatment with the nitrogen mustards, 15 were mosaics. A particularly interesting case of mosaicism for a visible mutation occurred among the progeny of ♂♂ treated with nitrogen mustard A and subsequently mated to attached-X ♀♀. One of the sons was both somatically and gonadically a mosaic for two different alleles at the white locus. This case has been discussed elsewhere (Auerbach, 1946).

## Dominant lethality.

♂♂ treated with nitrogen mustards showed a marked decrease in fertility. No records were taken of the hatchability of eggs after treatment of the father with allyl isothiocyanate. For all remaining substances hatchability of eggs inseminated by treated ♂♂ was normal; but with higher doses many ♂♂ failed to inseminate their mates.

## Chromosome breaks

Tests for the production of large deletions were carried out with the nitrogen/

nitrogen mustards and with mustard oil. The results are summarized in Table II.

Table II.

Tests for the production of chromosome breaks by chemical substances (not including mustard gas). Treated wild-type (Florida-4) ♂♂ were mated to attached-X ♀♀ carrying the marker genes y, v, and f, and the $F_1$ was examined for daughters in which one or two of the marker genes were covered by the presence of a deleted fragmented of the paternal X-chromosome.

| Substance | % of sex-linked lethals in simultaneous ClB - test. | No. of $F_1$ ♀♀ | | |
|---|---|---|---|---|
| | | normal diploid | triplo-X | hyperploid for a deleted X. |
| Nitrogen mustard A | 5.4 | 2806 | 296 | 8 |
| Nitrogen mustard B. | 6.2 | 980 | 50 | 1 |
| Nitrogen mustard C . | 8.5 | 834 | 4 | 1 |
| Mustard oil | 2.2 | 1768 | 144 | 1 |

The spontaneous occurrence of chromosome re-arrangements is so extremely rare that the oc currence of 8 of them - at least 7 of which occurred singly - among about 3000 daughters of ♂♂ treated with nitrogen mustard A can be taken as proof for the ability of this substance to produce chromosome breaks and re-arrangements. Nitrogen mustards B and C were tested on too small a scale to make the results equally convincing. The one apparent deletion in the progeny of ♂♂ treated with B was in reality a translocation through which the left end of the treated X-chromosome had become attached to the Y-chromosome. This re-arrangement must thus have arisen in a premeiotic male germ cell and was not a deletion sensu stricto. In general, the genetical method used does not allow to differentiate between translocations of this type and true deletions, except by further breeding tests; but since both types of re-arrangements require two independent chromosome breaks for their production, such differentiation is unnecessary/-

unnecessary in experiments in which the capacity of an agency to break the chromosomes is being tested. Incidentally, it is of interest to note that the X-Y translocation behaved in the same way as has been described previously by Philip (1934), Morgan (1938), and Pontecorvo (personal communication) for similar translocations: in oocytes which contained both a normal Y and the Y with the translocated fragment of the X, the latter segregated preferentially into the same cell as the attached X's. A second translocation, between X and III, was found in the ClB test carried out with nitrogen mustard B. The occurrence of 2 translocations in the two parts of the same experiment strongly suggests that this substance, too, can produce chromosome breaks. After treatment with nitrogen mustard C, 1 deletion was found in over 800 daughters. This result, although it is not sufficient to prove that the substance produces chromosome breaks, is at least suggestive, especially as the small percentage of triplo-X ♀♀ in this experiment indicates that the culture conditions were not favourable for the survival of unbalanced types. On the other hand, the occurrence of one deletion among almost 2000 daughters of ♂♂ which had been treated with mustard oil is not sufficient to prove that mustard oil can break the chromosomes.

## Discussion

The results presented here show that substances which have a high power of penetration and cause fixation of protoplasm and vesication are not necessarily mutagens. Thus osmic acid and picric acid penetrate well and fix protoplasm, and lewisite causes vesication, yet none of these appears to produce a selective action on the chromosomes. However, the possibility must be considered, especially in the case of lewisite, that a mutagenic substance may, through some unrelated toxic action, kill the flies at concentrations which are below the threshold for its action on the chromosomes. In such cases, different

methods/-

methods of application, e.g. treatment of free sperm with subsequent artificial insemination, or Hadorn's (1946) new technique of treating explanted ovaries, might reveal mutagenic possibilities which could not be detected by our methods.

It is of interest that all the substances which have been shown to be effective in the present investigation share certain characteristics of chemical structure. All of them seem to possess (1) an atom with unsaturated valencies, namely S in mustard gas and in $O(CH_2.CH_2.S.CH_2.CH_2.Cl)_2$, and N in $CH_3.N(CH_2.CH_2.Cl)_2$ and in $N(CH_2.CH_2.Cl)_3$, and (2) at some point at the periphery of the molecule, two or more activating atoms or atom groups, namely Cl in mustard gas and the three other highly effective substances used in the present investigation. It is likely that activation of this type will facilitate chemical reactivity through primary formation of addition compounds. It will be noticed that allyl isothiocyanate, which is a definite though weak mutagen, and chloracetone and dichloracetone, both of which may be weak mutagens, possess similar chemical characteristics: in allylisothiocyanate and S-atom can be activated by the double bond of the allyl group, whereas in the two acetone compounds chlorine and O play the roles of activator and activated atom respectively. It has been claimed (Lobashov 1937) that ammonia, which also possesses an unsaturated N, but no activating atom, increases the mutation rate; the present data, while they do not support this claim, are not opposed to the possibility that ammonia possesses a very slight mutagenic action. It seems promising to undertake systematic tests of substances which, in their chemical structure, are intermediate between $NH_3$ and the highly effective $N(CH_2.CH_2.Cl)_3$; substances such as the ethyl amines and others built on a similar pattern. These will, it is hoped, be tested in the near future.

It is not, however, suggested that the chemical characteristics of the

mustard group are the only ones which can endow a substance with mutagenic activity. Indeed, the recent results with phenol (Stubbe, 1940, Hadorn and Niggli, 1946) indicate that such is not the case, and it may well be found ultimately that a number of chemical substances, varying widely in constitution, can produce the same type of action on the nucleus. Of course, the slighter their effect, the more difficult will it be to detect it. For the smaller the expected difference between treated and control series, the larger will be the scale on which work has to be carried out in order to give a statistically satisfactory result; the more important, too, is the rigid control of environmental conditions and of physiological and genotypical similarity between treated and control material. Choice of a test organism which is more sensitive and at the same time more easily reared than Drosophila may make it possible to overcome these difficulties to a certain extent; but induced mutation rates which fall within the range of spontaneous mutability may elude detection even with such a material. For in this region even a statistically significant result must be regarded with suspicion until several experiments, each with its own separate control, have given positive results. These precautions have all too frequently been neglected in previous investigations with chemical substances.

A number of suggestions have been made to account for the origin of spontaneous mutation. Cosmic radiation and radio-active substances in the soil cannot be responsible for all of them, as has been shown by Muller and Mott-Smith (1930). Timoféeff-Ressovsky, Zimmer and Delbrück (1935) have suggested that mutations are produced when the random temperature fluctuations near a gene overstep a certain energy threshold, and this has been generally accepted by geneticists. The present work, however, suggests that spontaneous mutations may to some extent be due to naturally occurring chemical substances, either/-

either introduced from the outside, or - more likely - arising as metabolites in the organism itself. Such a hypothesis is attractive, since it might provide a mechanism for adjusting mutability to evolutionary requirements. It would also satisfactorily account for the fact that spontaneous mutability is influenced by agencies which alter metabolism, e.g. age (Muller 1946: Lamy, in the press), sex, (Auerbach 1941, Zamenhof 1945) and starvation (Olenov 1939). Moreover genes which influence the mutation rate of other genes (Demerec 1937, Rhoades 1938, Mampell 1943) may have a similar mode of action. Incidentally it must be considered that substances which produce a slight effect on the mutation rate may do so, not by a direct action on the chromosomes, but indirectly by interfering with metabolism.

In recent years, there has been much discussion as to whether gene mutations differ essentially from chromosome re-arrangements. The original data on the effects of ultra-violet rays seemed to indicate that here was an agency which produced gene mutations, but neither chromosome re-arrangements nor small deficiencies in appreciable numbers (Stadler 1939, Muller and Mackenzie, 1939, Mackenzie and Muller 1940). Subsequent work, however, (Stadler 1941, Slizynski 1942) has shown that ultra-violet radiation, too, is capable of producing both minute and large re-arrangements. The same is true of mustard gas (Auerbach and Robson, 1946, Slizynski and Slizynska, in the press) and, as the data here presented show, of two, probably three, other highly potent mutagens. The weak mutagen, allyl isothiocyanate, in a dose producing 2.2% sex-linked lethals, apparently gave a negative result for deletions. This cannot, however, be considered as conclusive, since it is possible that the frequency of chemically induced large re-arrangements falls off as the square of the dose, as has been found for low doses of X-rays (Muller, 1940).

Experiments/-

Experiments on a very much larger scale than the present one may therefore be required to detect a significant increase in the frequency of re-arrangements after treatment with allyl isothiocyanate. The same holds for other weak mutagens. Work on plant chromosomes, in which breaks are more easily produced and detected than in Drosophila, might provide a better method for testing such substances.

Of particular interest would be a chemical mutagen which does not produce minute re-arrangements; for it is these which seem to pass imperceptibly into gene mutations and are believed by some workers to be responsible for all gene mutations. Data obtained by Slizynski and Slizynska (in the press) suggest that the percentage of small deficiencies among sex-linked lethals is the same whether these lethals have been induced by X-radiation, ultraviolet radiation, or mustard gas. It would be interesting to carry out, by the salivary gland technique, tests for small deficiencies in sufficiently large samples of lethals induced by weak chemical mutagens.

One of the properties of mustard gas which distinguishes its mutagenic action from that of X-rays is the high frequency of mosaics in the $F_1$ of treated ♂♂ (Auerbach, in the press). The data presented here show that mosaics also form a high proportion of those visible mutants which have been produced by treatment of ♂♂ with nitrogen mustards. Certain results obtained with nitrogen mustard A suggest, moreover, that gonadic mosaics may also be comparatively frequent. It would, thus, seem that the production of a large production of mosaics, including gonadic mosaics, is characteristic of chemical mutagens.

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## Summary

The genetic effects of a number of substances related chemically or pharmacologically to mustard gas were investigated on Drosophila melanogaster. Three of these were found to be highly active mutagens. They all belong to the class of the so-called nitrogen mustards. Their chemical formulae are: $CH_3.N(CH_2.CH_2.Cl)_2$, $N(CH_2.CH_2Cl)_3$, and $O(CH_2.CH_2.S.CH_2.CH_2Cl)_2$. The first of these substances was also shown to produce chromosome re-arrangements. The second and third very probably have the same capacity, although this was not definitely established.

Allyl isothiocyanate, mustard oil, has a definite, though weak capacity to induce mutations. No clear evidence that it can cause chromosome re-arrangements was obtained.

Chloracetone and dichloracetone gave doubtful results consistent with the possibility that these substances have a weak mutagenic action. Experiments with ammonia do not exclude the possibility that it may have a very slight effect on mutation rate. The same is true of osmic acid and picric acid.

Lewisite gave entirely negative results at doses which killed the majority of the treated flies.

It is suggested that the substances found to be effective owe this property to a specific chemical configuration which is discussed.

As after mustard gas treatment, mosaics appear to form a high proportion of mutants in the progeny of males which have been treated with the other highly effective substances.

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# The Problem of Chromosome Re-arrangements in Somatic Cells of *Drosophila melanogaster*

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# XVI.—The Problem of Chromosome Re-arrangements in Somatic Cells of *Drosophila Melanogaster*.

By **C. Auerbach**, Ph.D., Institute of Animal Genetics. *Communicated by* Dr A. W. Greenwood. (With Two Text-figures.)

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## Introduction

The mechanism by which irradiation produces gross re-arrangements of chromosomes (inversions, translocations, deletions) is now generally believed to occur in two steps: (*a*) breakage of one or more chromosomes, yielding two or more points of breakage; and (*b*) reunion of broken ends in a novel combination. Thus the frequency with which a given treatment produces re-arrangements depends not only on the number of breaks produced, but on the circumstances under which reunion takes place. In *Drosophila* the fertilized ovum seems to offer particularly favourable conditions for breaks produced in the mature spermatozoon to join up into new combinations. In immature germ-cells, on the other hand, re-arrangements are much less readily produced by irradiation (Muller, 1930; Shapiro and Neuhaus, 1933; Shapiro, 1937; Glass, 1939). Evidence for the occurrence of spontaneous or induced re-arrangements in somatic cells of *Drosophila* is very scanty. L. V. Morgan (1939) reports on a spontaneous translocation which occurred in one part only of a salivary gland and therefore must have arisen during the divisions of the gland precursor cells. Slizynski (unpubl.) obtained cytological proof of the production of deletions in irradiated or chemically treated salivary cells of embryos. The bulk of the evidence for induced re-arrangements in somatic cells of *Drosophila* appeared to come from the work of Patterson (1929 *a*, *b*) who, by X-raying embryos and larvæ which were heterozygous for recessive sex-linked markers, obtained great numbers of flies in which certain regions of the body exhibited the marker genes, as a result of what was then assumed to be an induction of deletions or deficiencies in the treated chromosomes carrying the wild-type allelomorphs. However, the validity of this interpretation was laid open to doubt by Stern's (1936) discovery that somatic crossing-over may give similar results. Muller, when discussing this point in his review at the Cold Spring Harbor Symposium, 1941, arrived at the conclusion that "when re-examined these data show little or no sign of the production of deletions or of terminal deficiencies in the soma, the phenomena described under this heading giving every indication of being in the majority of cases due to somatic crossing-over."

A distinction between the results of somatic deletions and somatic crossing-over is possible only when each of the two homologous chromosomes carries at least one suitable recessive marker so that crossing-over can lead to twin spots, *i.e.* adjoining areas each of which exhibits one of the marker genes. Since Patterson's work on mosaics was carried out before Stern's paper on somatic crossing-over appeared, only some of his experiments happened to be of the type described above, so that the material on which a re-examination of his data from the new point of view can be carried out is not very extensive. The present paper supplements his data by an analysis of a large number of mosaic spots which were produced by chemical treatment (Auerbach, 1943; Auerbach and Robson, 1944) of *Drosophila melanogaster* eggs.

In the first group of experiments, the ♀♀ examined for mosaic spots were of one of the following genotypes: *y w sn/loz* and *y w loz/sn* (*y* = yellow, *w* = white, *sn* = singed, *loz* = lozenge; all genes sex-linked). Table I gives the pooled results from all experiments in this group, as well as of a few others of similar kind. It shows the high efficiency of chemical treatment in producing mosaics.

Three main mechanisms for the origin of mosaic spots can be considered, namely:

(*a*) Loss of one entire chromosome, due either to abnormal chromosome behaviour at mitosis or to sister-chromatid reunion following the production of a single break. Chromosome

TABLE I.—FREQUENCY OF MOSAICS AMONG HETEROZYGOUS ♀♀ WHICH HAD BEEN CHEMICALLY TREATED AS EMBRYOS

| Series | Genotype of ♀♀ | Total ♀♀ | Mosaic Individuals | | No. of Mosaic Spots | Spots per ♀ |
|---|---|---|---|---|---|---|
| | | | No. | Per cent. | | |
| Treated . . | *y w sn/loz* and *y w sn/+* | 318 | 117 | 37 | 189 | ·6 |
| Treated . . | *y w loz/sn* | 266 | 135 | 51 | 159 | ·6 |
| All treated . . | combined | 584 | 252 | 43 | 348 | ·6 |
| Control . . | *y w sn/loz* | 159 | 1 (?) | < 1 | 1 (?) | < ·01 |
| | *y w loz/sn* | 154 | 4 | 2·5 | 4 | ·03 |
| All controls . . | combined | 313 | 4–5 | 1·5 | 4–5 | ·02 |

*Note.*—The question mark refers to a spot consisting of a single bent bristle which may or may not have been *sn*.

loss exposes all the marker genes of the homologous chromosome. Additional evidence of the loss of an X-chromosome may be obtained when spots in suitable parts of the body are large enough to exhibit ♂ characteristics; but these conditions were not fulfilled for any of the mosaics.

(*b*) Deletion, either terminal or interstitial. This may uncover any one or more of the marker genes.

(*c*) Somatic crossing-over. The phenotypical effect of this mechanism depends on the distribution of the marker genes, and on the region in which crossing-over takes place.

As a fourth possible mechanism somatic gene-mutation might be considered; but data obtained from treated ♂♂, in which all mosaic spots must be due to mutation, indicate that mutation probably contributes less than 1/20th of the number of observed spots.

Three genotypes were used, namely *y w sn/loz*, *y w loz/sn*, and *y w sn/y w f Dp(scy +)* (*f* = forked, *s* = scute). The results obtained with each genotype will be discussed separately.

### *Experiment I. Genotype of treated ♀♀ y w sn/loz*

Somatic crossing-over gives the results represented in the upper half of fig. 1. For the sake of simplicity, the small distance between *sn* (locus 21) and *loz* (locus 27·7) has been disregarded, as well as the even smaller distance between *y* (0) and *w* (1·5), and the chromosome has been subdivided into a distal region I extending from *y* or *w* to *sn* or *loz*, and a proximal region II. Table II classifies the results which would be produced by the three mechanisms.

These expectations may be compared with the actual data which are summarized in Table III.

In this experiment, a recognition of somatic crossing-over by the occurrence of twin spots is possible only in the eyes. About one-half of the spots in the eyes are twins, the remainder are white without lozenge or lozenge without white. Table II shows that these types of spots can arise in different ways: by single crossing-over in the distal region (white spots), by double crossing-over (lozenge spots), by chromosome loss, or by deletion. However, comparison of Table III with the upper row of Table II shows that bristle spots which must be due to either distal or double crossing-over or to deletion, namely the yellow and the singed spots, are extremely rare. The rarity of distal cross-overs is in keeping with Stern's observation that somatic crossing-over takes place preferentially near the spindle fibre, although to the left of the *bb* locus. Since there is no ground for the assumption that conditions in the developing eye disc should favour either the rare types of crossing-over or deletions, it does not seem possible to interpret the *w* and *loz* spots as having arisen by one of these mechanisms. According to Table II, the only other alternative is chromosome

TABLE II.—TYPES OF MOSAIC SPOTS EXPECTED IN *y w sn/loz* ♀♀ ACCORDING TO THE FOLLOWING THREE DIFFERENT ASSUMPTIONS ABOUT THEIR ORIGIN: (A) CHROMOSOME LOSS, (B) DELETION, (C) SOMATIC CROSSING-OVER (*cp.* FIG. 1)

| | (A) Chromosome Loss | (B) Deletion | (C) Crossing-over in— | | |
|---|---|---|---|---|---|
| | | | I | II | I and II |
| Bristles . . | *y sn* | *y*<br>*sn*<br>*y sn* | *y* | *y sn* | *sn* |
| Eyes . . | *w*<br>*loz* | *w*<br>*loz* | *w* | *w* and *loz* | *loz* |

*Note.*—"*w* and *loz*" signifies a twin spot, one member of which is *w* and the other *loz*.

TABLE III.—FREQUENCIES OF THE DIFFERENT TYPES OF MOSAIC SPOTS IN *y w sn/loz* ♀♀

| Type of Spot . . | *y sn* | *y* | *sn* | *w* and *loz* | *loz* | *w* |
|---|---|---|---|---|---|---|
| No. of spots obtained . | 58 | 1<br>1 (?) | 1 (?) | 39 | 24 | 21 |

*Note.*—The question marks refer to small spots of doubtful phenotype.

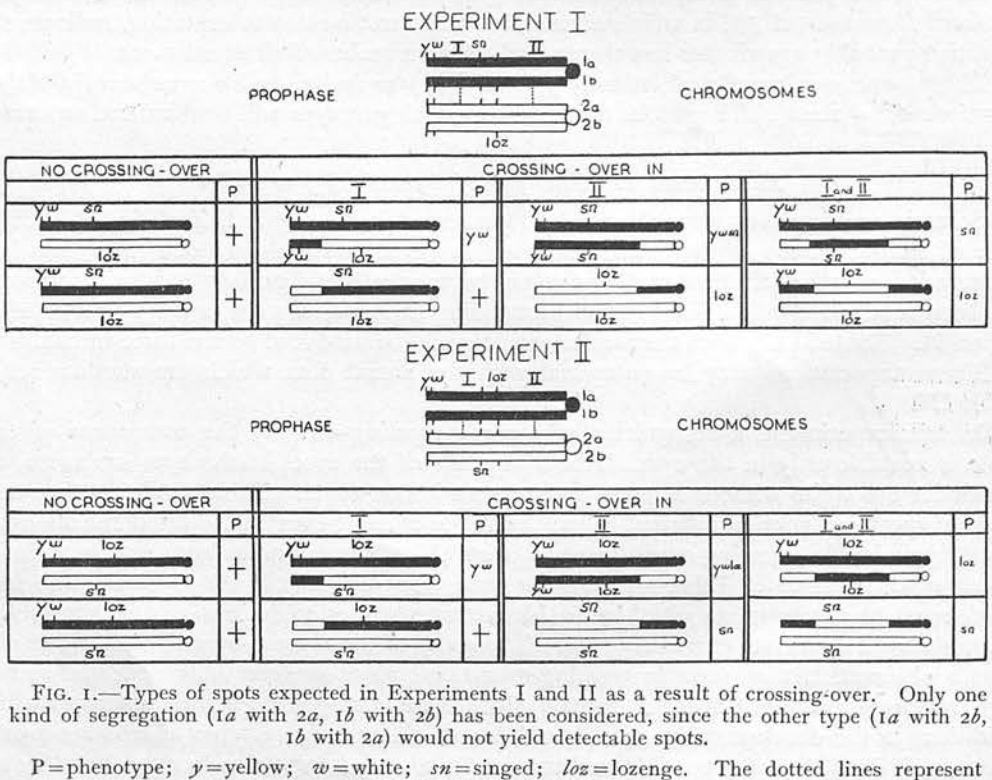


FIG. 1.—Types of spots expected in Experiments I and II as a result of crossing-over. Only one kind of segregation (1*a* with 2*a*, 1*b* with 2*b*) has been considered, since the other type (1*a* with 2*b*, 1*b* with 2*a*) would not yield detectable spots.

P=phenotype; *y*=yellow; *w*=white; *sn*=singed; *loz*=lozenge. The dotted lines represent points of crossing-over.

loss; but the possibility has also to be considered that an apparent single spot in reality may be one member of a pair of twin spots, the other twin by some accident of development not having reached the surface, or having failed to develop altogether. The occurrence of such incomplete twin spots has been postulated also by Stern, especially for cases where the mosaic area is small, a condition which—for reasons which will be discussed elsewhere—was usually fulfilled in the present experiments. Patterson's data, too, on re-examination from the new point of view, suggest the frequent occurrence of incomplete twin spots. These considerations show that the unpaired eye spots may be explained without recourse to the assumption of induced chromosome losses; yet it seemed desirable to carry out a special experiment in which the occurrence of losses of whole chromosomes could be tested. Before the discussion of this experiment, a brief report will be given on the results of the second experiment in which *y w loz/sn* embryos were treated.

### *Experiment II. Genotype of treated ♀♀ y w loz/sn*

The results of somatic crossing-over are represented in the lower half of fig. 1. Table IV shows which types of spots are to be expected on various assumptions about their origin. The observed data are presented in Table V.

TABLE IV.—TYPES OF MOSAIC SPOTS EXPECTED IN *y w loz/sn* ♀♀ ACCORDING TO THE FOLLOWING THREE DIFFERENT ASSUMPTIONS ABOUT THEIR ORIGIN: (A) CHROMOSOME LOSS, (B) DELETION, (C) SOMATIC CROSSING-OVER (*cp.* FIG. 1)

| | (A) Chromosome Loss | (B) Deletion | (C) Crossing-over in— | | |
|---|---|---|---|---|---|
| | | | I | II | I and II |
| Bristles . . | *y*<br>*sn* | *y*<br>*sn* | *y* | *y* and *sn* | *sn* |
| Eyes . . | *w loz* | *w*<br>*loz*<br>*w loz* | *w* | *w loz* | *loz* |

*Note.*—"*y* and *sn*" signifies a pair of twin spots, one member of which is *y* and the other *sn*.

TABLE V.—FREQUENCIES OF THE DIFFERENT TYPES OF MOSAIC SPOTS IN *y w loz/sn* ♀♀

| Type of Spot . . | *y and sn* | *y* | *sn* | *w loz* | *w* | *loz* |
|---|---|---|---|---|---|---|
| No. of spots obtained . | 44 | 23 | 57 | 23 | 6<br>1 (?) | 3<br>2 (*w loz*?) |

*Note.*—The question marks refer to cases in which it was difficult to decide whether 1 or 2 lighter coloured facets were proper whites.

Tables IV and V tell the same story as did Tables II and III. Here it is the bristle spots which, if they have been produced by somatic crossing-over, should occur as twin spots. Again single spots are very numerous, and again comparison with the data on eye spots shows that distal or double crossing-over or deletion can at best account for only a small proportion of them. The remainder must be due to either chromosome loss or to failure of one partner of a twin spot to become manifest. A special feature of this experiment is the preponderance of *sn* over *y* single spots. This may be due to either of two causes: (1) lowered viability of homozygous *y w loz* as compared with homozygous *sn* cells, (2) the greater ease with which individual yellow bristles may escape detection, especially on the abdomen, or in regions of the thorax which are covered by microsetæ only.

*Experiment III. Genotype of treated ♀♀ y w sn/y f.y+*

For the detection of possible chromosome losses under the influence of chemical treatment, *y w sn* ♀♀ were mated to ♂♂ of the following genetical constitution: their X-chromosome is **V**-shaped, the left arm carrying the genes *y*, *w*, and *f*, and the right—longer—arm consists of heterochromatin, derived mainly from $Y^S$, to which at its distal end a duplication of the tip of the X, carrying *sc* and the normal allelomorph of *y*, is attached. (For description and origin of this chromosome see Pontecorvo, 1940.) The full genetical formula of these ♂♂ is thus *y w f/Dp(sc y+)*. Daughters produced by the mating of these ♂♂ to *y w sn* ♀♀ are wild-type apart from white eyes, since *y* is covered up by *y+* in the duplication of the paternal *X*-chromosome. The essential marker genes for the present problem are *y* and *sn*: loss of the whole paternal chromosome will result in mosaic spots in which both *y* and *sn* are exposed, whereas crossing-over between *sn* and the spindle-fibre attachment will uncover *sn*, the *y+* in the duplication not having been removed from the cell. The genes *w* and *sc*, as irrelevant for the present purpose, have not been entered in the diagram, and for the sake of simplicity the chromosome is designated by the abbreviated formula *y f.y+*. In fig. 2 the results expected from different types of crossing-over are set out diagrammatically. Four chromosome regions have been distinguished, namely: I from *y* to *sn*, II from *sn* to *f*, III from *f* to the spindle fibre attachment, and IV the right arm of the *yf.y+* chromosome. As can be seen from fig. 2, crossing-over between the heterochromatin in region IV and the proximal heterochromatin of either a sister chromatid (last two rows of fig. 2) or of the *y w sn* chromosome (two preceding rows of fig. 2) leads to *y* spots. The same would be true of spots arising by crossing-over between the *sc y+* end of the *y f.y+* chromosome and its homologous region on the *y w sn* chromosome; but owing to the small size of the duplication this type of crossing-over will be extremely rare. Table VI gives the types of spots expected on different assumptions about their origin.

TABLE VI.—TYPES OF MOSAIC BRISTLE SPOTS EXPECTED IN *y w sn/y f.y+* ♀♀ UNDER THE FOLLOWING THREE DIFFERENT ASSUMPTIONS ABOUT THEIR ORIGIN: (A) CHROMOSOME LOSS, (B) DELETION, (C) SOMATIC CROSSING-OVER

| (A) Chromosome loss | (B) Deletion | (C) Crossing-over in— | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | II and III | IV |
| *y sn* *f* | *y* *sn* *f* | .. | *sn* | *sn* and *f* | *f* | *y* |

*Note.*—"*sn* and *f*" signifies a pair of twin spots one member of which is *sn* and the other *f*.

Table VII presents the results obtained in Experiment III.

TABLE VII.—FREQUENCIES OF THE DIFFERENT TYPES OF MOSAIC SPOTS IN *y w sn/y f.y+* ♀♀

| Type of Spot . . . | *sn* (or *f*) | *y* | *y sn* |
|---|---|---|---|
| No. of spots obtained . | 33 | 6 | 0 |

*Note.*—No attempt was made to distinguish between *sn* and *f* spots, so that the spots scored as *sn* may include also *f* spots and (*sn* and *f*) twin spots.

The salient point of this experiment is that in a total of 39 spots not a single *y sn* spot was obtained. This shows that loss of a whole chromosome in cells of chemically treated embryos, if it occurs at all, must be very rare. As Patterson (1930) has shown, the same is true for irradiated embryos. Application of this result to the interpretation of Experiments I and II leads to the conclusion that there, too, single spots are due not to chromosome loss, but mainly or wholly to failure of one partner of a twin spot to manifest itself. Experiment III provides

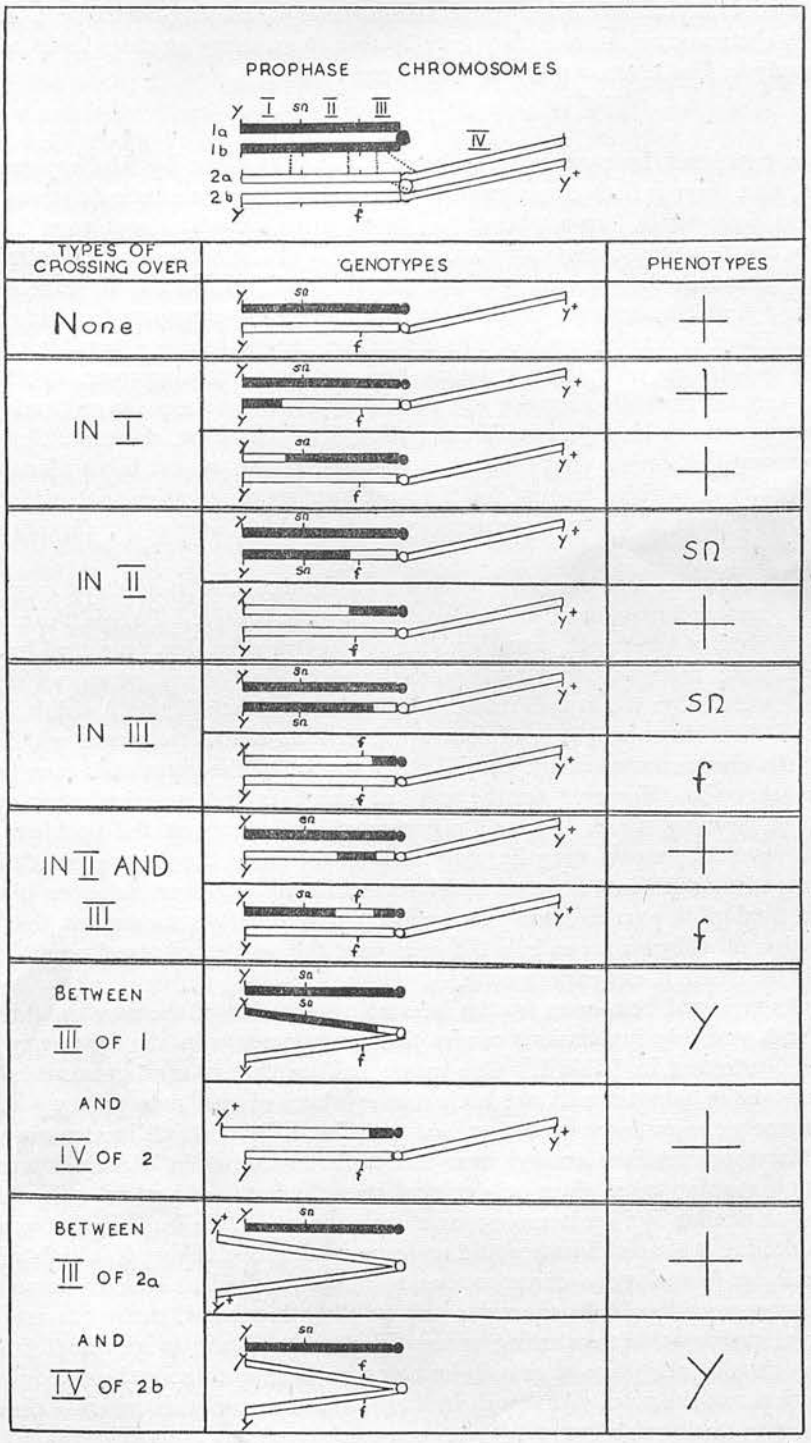


FIG. 2.—Types of spots expected in Experiment III as a result of crossing-over. Only one kind of segregation (1*a* with 2*a*, 1*b* with 2*b*) has been considered, since the other type (1*a* with 2*b*, 1*b* with 2*a*) would not yield detectable spots.

P = phenotype; *y* = yellow; *sn* = singed; *loz* = lozenge; *f* = forked. The dotted lines represent points of crossing-over.

no more evidence for the production of somatic deletions than the preceding experiments. The 6 yellow spots in Table VII may have arisen through crossing-over within the right arm of the $y\,f.y^+$ chromosome, or else they may be due to mottling at the $y$ locus, which occurs quite frequently in flies heterozygous for this chromosome.

## Conclusion

The data presented here agree entirely with the statement by Muller, quoted in the introduction, that there is little or no proof for the production of somatic deletions or terminal deficiencies in *Drosophila*. In a total of 343 spots, there are only about 10 which cannot be explained by single somatic crossing-over, and for all of these there remains an alternative explanation: either double crossing-over, or crossing-over within the large heterochromatic region of the $y\,f.y^+$ chromosome, or mottling for $y$ in ♀♀ heterozygous for this chromosome. As a matter of fact, in experiments of this kind it will never be quite possible to distinguish between the results of interstitial deletion and multiple crossing-over. Therefore these experiments can never really disprove the possibility that some mosaic spots may be due to induced chromosome re-arrangements in somatic cells. However, data like those presented here go far towards showing that such an occurrence at the utmost takes place very rarely, certainly not often enough to account for the large number of recessive spots which have been found after irradiation of heterozygous embryos, and which have originally been attributed to deletions.

It might be objected that chemical treatment is fundamentally different from irradiation and in its very nature incapable of producing chromosome breaks. To this it can be answered that the same kind of treatment, when applied to ♂ germ cells, has produced both deletions and translocations; moreover, that ♂♂ from the same batches of treated eggs which provided the mosaic ♀♀ were subjected to a C/B test and produced 18 lethals and 8 visible mutations in 708 treated X-chromosomes. It is of course conceivable that a treatment which is capable of breaking the chromosomes in the special environment of the germ cells may be unable to do so in somatic cells. However, for the point at issue it is immaterial whether or not this is the case. The bearing which the results presented here have on the problem of somatic chromosome re-arrangements may be expressed as follows: the data show definitely that large numbers of mosaics may be produced by somatic crossing-over, deletions playing at the best a quite negligible part in their production. If one wishes to assume that mosaicism after irradiation of embryos arises in a different way, the burden of proof would lie with new and specially designed X-ray experiments.

The results reported here open up the interesting question of the way in which chemical treatment (and probably irradiation) causes the great increase in the frequency of somatic crossing-over illustrated in Table I. One may speculate that re-arrangements in germ cells and crossing-over in somatic cells are both consequences of the same primary action of the treatment, namely chromosome breakage, but with the difference that in somatic cells, owing to the peculiar topographical arrangement of the chromosomes in higher Diptera (somatic pairing), a new reunion takes place only or preferentially between broken ends which happen to be placed at similar loci on homologous chromosomes. This implies that practically all breaks not fulfilling these conditions would undergo restitution. Since it is highly improbable for two breaks on homologous chromosomes to have occurred at strictly homologous loci, unequal crossing-over should be the rule. If, on the other hand, treatment acts merely by intensifying a spontaneously occurring process similar in nature to germinal crossing-over, the exchange should take place at exactly homologous loci, and unequal crossing-over should be the exception, not the rule. At first sight it appears as though this criterion should provide an easy means to distinguish between the two assumptions. A homozygous $w^a$ strain seems a suitable object for the test. When ♀♀ of this constitution are treated as embryos or larvæ, the results of unequal crossing-over about the $w^a$ locus should be apparent by the occurrence of twin spots, one member of which would be darker than its surroundings ($w^a/w^a Dp(w^a)$), while the other would be lighter ($w^a/Df(.w^a)$). No such result was obtained in an experiment carried out by Patterson (1929). It has to be considered, however, that somatic crossing-over has a very marked tendency to occur in the proximal region of the X-chromosome, according to Stern (1936) between *f* and *bb*. Therefore, in the great majority of cases, crossing-over,

whether equal or unequal, must have occurred to the right of $w^a$, leaving the constitution of the resulting cells unaltered in respect of the $w^a$ locus. A clearer result might be expected from the use of a $w^a$ stock in which the $w^a$ gene has been transposed by inversion into the proximal region of the X-chromosome. Such a test is now in preparation.

Finally, it has to be considered whether the extreme rarity of chromosome re-arrangements in somatic as compared with gametic cells is a peculiarity of *Drosophila* and probably other higher Diptera, or whether it holds also for other animals. It is very unlikely that somatic crossing-over should play a rôle in cells in which the chromosomes do not show somatic pairing; but it still may be true that many more broken ends undergo restitution in somatic cells than in germ cells. There can be no doubt that a certain amount of re-arrangement does take place after irradiation of immature germ cells and somatic cells of various animals (see, *e.g.*, Carlsson (1941) and White (1935) on grasshoppers, Pontecorvo (in press) on lice); but the data which so far have been obtained are small and do not allow of a quantitative comparison between gametes, immature germ cells, and ordinary somatic cells in animals other than *Drosophila*.

SUMMARY

Chemical treatment of ♀ embryos heterozygous for suitable marker combinations leads to a high occurrence of mosaicism, about 40 per cent. mosaic individuals having been obtained in the present experiments. There is no evidence that any of the produced mosaic spots arose by either deletion or terminal deficiency or loss of a whole chromosome. Somatic crossing-over appears to have been the only, or at least by far the most important, source of mosaicism. It is inferred that also after irradiation of embryos, mosaicism is solely or almost solely due to somatic crossing-over. The suggestion is put forward that the mechanism of breakage and reunion which underlies chromosome re-arrangement in germ cells may be responsible for the apparent crossing-over in somatic cells of higher Diptera.

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# Chemically Induced Mosaicism in *Drosophila melanogaster*

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# XXV.—Chemically Induced Mosaicism in *Drosophila melanogaster*.

By **Charlotte Auerbach,** The Institute of Animal Genetics, University of Edinburgh. *Communicated by* Dr A. W. Greenwood. (With One Text-figure.)

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Muller's classical discovery in 1927 of the mutagenic action of X-rays has provided an extremely useful tool for studying the nature of gene mutation and chromosome breakage. It has always been realized that if, in addition, chemical substances were found capable of producing mutations and/or chromosome breaks a further important step forward in the analysis of the mutation process would follow. The search for such mutagenic substances has therefore been going on in various laboratories for some ten years, until recently, however, without definitely positive results. Only during the last few years has it been shown that certain chemical substances, such as mustard gas ($\beta\beta'$-dichlordiethylsulphide, $(ClCH_2 \cdot CH_2)_2S$), may be as effective as X-rays in producing mutations and chromosome breaks (Auerbach, 1943; Auerbach and Robson, 1944; Auerbach, 1945; Auerbach and Robson, 1946). Indeed, the similarities between the results of these two types of treatment are impressive. In the course of more than four years during which work of this kind has been carried out by the author, only few differences between the genetical results of chemical treatment and of irradiation have come to light. Particular interest attaches to these dissimilarities, because they, rather than the many similarities, may throw some new light on the process of mutation and through this on the nature of the gene. One of the few striking dissimilarities is the high frequency of certain types of mosaics which can be produced by chemical treatment. It is the object of the present paper to summarize the data on chemically induced mosaicism, contrast them with similar data from X-ray experiments, and discuss the similarities and differences from the point of view of the mechanism of induced mutation.

Mosaicism is to be understood as the co-existence, in one and the same individual, of tissues of different genetical constitution. In *Drosophila* two different methods of irradiation have been found to yield mosaics:

(1) Irradiation of embryos and larvæ results in mosaics in which the size of the genetically altered area depends on the stage at the time of treatment, younger stages giving rise to larger aberrant areas than older ones (Patterson, 1929).

(2) Irradiation of adult ♂♂ results in progenies which usually contain a certain proportion of individuals which are mosaics of an approximately half-and-half type for an induced mutation or chromosome aberration.

Both methods have proved successful also with chemical treatment, *i.e.* exposure to mustard gas or similar substances. There exist, however, quantitative differences in the effects of the two types of treatment. (1) In mosaics from chemically treated embryos, the aberrant spots tend to be smaller than after irradiation. (2) The progeny of chemically treated ♂♂ contains a larger percentage of mosaic individuals than the progeny of irradiated ♂♂.

## 1. Mosaics from Treated Embryos

When ♀ embryos which are heterozygous for recessive sex-linked markers are given sufficient chemical treatment, a high percentage (about 40 per cent. with the doses here used) of the developing flies exhibit one or more of the marker genes in one or more areas of their body. These experiments have been described previously (Auerbach, 1945), and it has been shown that the main cause for this type of mosaicism is somatic crossing-over.

A comparison of chemically produced mosaics with those obtained by Patterson (1929) by irradiation of embryos indicates a difference in the size of the individual spots after the two types of treatment. Eye-colour spots were used for the comparison. Since the set-up of Patterson's experiments was such that usually only one out of a pair of twin-spots could be detected, the size of a chemically produced, detectable twin-spot has been recorded as that of the larger one of its two components. Thus, the actual areas affected by somatic crossing-over were about twice as large as the values given in Table I, which summarizes the results of the comparison.

TABLE I.—SIZE OF ABERRANT AREAS IN THE EYES OF MOSAICS WHICH HAVE BEEN PRODUCED BY TREATING EGGS (*a*) WITH IRRADIATION, (*b*) CHEMICALLY

| Treatment | Author | Age in Hours at Treatment | No. of Mosaic Areas covering the following Number of Facets | | | | | Per cent. of Spots with less than 11 Facets |
|---|---|---|---|---|---|---|---|---|
| | | | 0–10 | 11–30 | 31–100 | 100+ | Total | |
| X-rays . | Patterson (1929) | 0–12 | 2 | 3 | 4 | 4 | 13 | 15 |
| | | 13–24 | 2 | 10 | 6 | 3 | 21 | 10 |
| | Auerbach (unpub.) | 0–20 * | 1 | 2 | 1 | 1 | 5 | 20 |
| X-rays . | All data combined | 0–24 | 5 | 15 | 11 | 8 | 39 | 13 |
| Chemical . | | 0–7½ | 5 | 2 | 2 | 0 | 9 | 56 |
| | | 0–20 * | 41 | 21 | 3 | 1 | 66 | 62 |
| Chemical . | All data combined | 0–20 | 46 | 23 | 5 | 1 | 75 | 61 |

* Since young embryos are extremely sensitive to chemical treatment, it is almost certain that the embryos in the (0–20) groups which survived the fairly strong treatment corresponded in age to Patterson's (13–24) rather than to his (0–12) group.

It will be seen from Table I that, whereas after irradiation of embryos the mosaic area usually covers more than 10 facets, after chemical treatment almost two-thirds of the spots were smaller than 11 facets; this is true even for flies which had been treated less than eight hours after laying of the egg, *i.e.* at an age when in Patterson's experiments only 2 out of 13 flies had mosaic areas of less than 10, but 4 of more than 100 facets. Similarly, in experiments in which yellow and singed were used as markers, 108 out of 207 bristle spots in both age groups comprised less than 3 setæ.

## 2. MOSAICS IN THE PROGENY OF TREATED ♂♂ ($F_1$ Fractionals)

When an egg is fertilized by a spermatozoon in which treatment has induced a mutation or chromosome re-arrangement, the resulting embryo would be expected to carry—and with a suitable genetical make-up to display—the abnormality throughout all its body. In reality, this is not always the case. It is a well-known fact that the $F_1$ of irradiated ♂♂ usually contains a certain proportion of individuals which are mosaics for a mutation or chromosome aberration. In the following, the term "fractional" aberrant, introduced by Muller (1928), will be used for mosaics of this origin. Those individuals which exhibit an inherited abnormality throughout all their body will in contrast be termed "complete" aberrants.

Fractionals may be of different kinds, depending on the nature of the induced change in the spermatozoon, and on the type of ♀♀ used in the experiment. Fig. 1 gives a diagrammatic representation of the main possible types of viable fractionals for the X-chromosome. It also includes those cases (A) in which mosaicism refers not to an aberration on the paternal chromosome, but is due simply to the loss of an apparently normal paternal

X at one of the cleavage divisions, resulting in a gynandromorph (AI) or a diplo-/triplo-X ♀ (AII). Classification of these types of mosaics as fractionals appears justified through the observation (Patterson, 1931, 1933) that X-ray treatment of ♂♂ increases the proportion of gynandromorphs among their progeny.

Loss of a deleted (B) or mutated (C) paternal X-chromosome at cleavage results in one possible type (*a* in fig. 1) of fractional aberrants. More frequently observed, however, is a different type of fractional, in which the non-aberrant part of the body carries a normal paternal X-chromosome (*b* in fig. 1). Various hypotheses have been put forward to account for this kind of mosaicism. Its designation in fig. 1 as "mosaicism due to chromatid restitution" is based on Muller's (1940) interpretation of X-ray fractionals (see Discussion). Alternative interpretations will be discussed at the end of this paper. It should, however, be noted that no matter what hypothesis is assumed about the origin of fractionals, their mutual relationship as indicated by the grouping in the diagram remains the same.

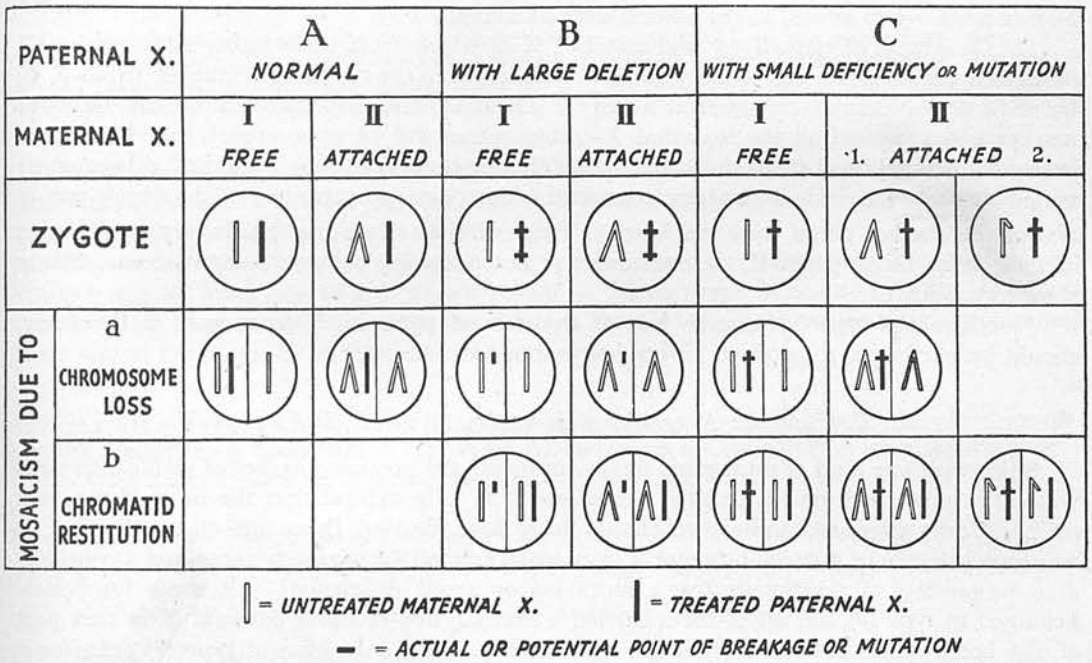


FIG. 1.—Main possible types of viable fractionals for the X-chromosome in the progeny of treated ♂♂.

*Note.*—The origin of mosaicism has been interpreted here on the basis of Muller's theory. The relationship between the various types of fractionals as indicated by their arrangement in the figure is, however, independent of any hypothesis concerning their origin.

Fractionals of many of the types represented in fig. 1 have been obtained after chemical treatment of ♂♂. As would be expected from considerations of viability, the only group—apart from gynandromorphs—which yielded sufficiently large data for a statistical evaluation were fractionals for mutations or small deficiencies (C). For the sake of completeness observations on the other groups will also be recorded.

### *A. Paternal X carries no apparent Abnormality*

Some gynandromorphs (I, *a*) have appeared in many experiments. An accurate comparison of their frequencies after chemical treatment and after irradiation has not yet been carried out; but there are no indications of their being more frequent after chemical treatment.

One diplo-/triplo-X ♀ (II, *a*) was found among 912 triplo-X daughters of attached-X ♀♀ by ♂♂ which had been given sufficient chemical treatment to produce from ·1 to 6 per cent. large deletions.

### *B. Paternal X carries a large Deletion*

I. Sons of treated ♂♂ and detached-X ♀♀ which from their father have received a deleted X-chromosome will be sterile (because of the absence of a Y-chromosome) and hyperploid; they may appear as intersexes. For the production of aberrants of this kind, treated wild-type or Bar ♂♂ were mated to *y w sn* ♀♀. Among altogether 9800 sons from such matings (three different experiments), there occurred 9 sterile *w sn* ♂♂ in which the maternal yellow gene was masked by the presence of a deleted paternal X carrying the wild-type allelomorph. In addition there occurred two fractionals of type (*b*), namely one gynandromorph in which the ♂♂ parts were *sn*, but neither *y* nor *w*, and a second one in which the *y w sn* portion of the body was distinctly intersexual.

Fractionals of type (*a*), due to loss of the deleted paternal X from part of the body, should be mosaics for *y w sn* and *w sn*. None were obtained.

II. Daughters of treated ♂♂ and attached-X ♀♀ which from their father have received a deleted X-chromosome can usually be distinguished from the normally occurring triplo-X ♀♀ by their lesser degree of manifest aneuploidy and by the fact that not all the recessive marker genes present on the maternal X-chromosomes will be uncovered. In a number of experiments, wild-type ♂♂ were treated with different mutagenic substances and subsequently mated to *y v f* ♀♀. Their progeny contained altogether 47 hyperploid ♀♀ in which one or two of the marker genes were displayed. Three of them were fractionals of type (*b*), part of their body being triplo-X, the remainder *f*, but not *y* (*v*, being non-autonomous, cannot show mosaicism).

Fractionals of type (*a*), due to loss of the deleted paternal X from part of the body, should be partly *y v f*, partly *f*. None were obtained.

### *C. Paternal X carries a Mutation or small Deficiency*

Since with any kind of mutagenic treatment by far the greatest number of viable aberrants will carry small deficiencies or gene mutations, it is only natural that the bulk of the data on chemically produced mosaicism should have been derived from this class. In fact, it was found that both ♂ and ♀ progeny of chemically treated ♂♂ regularly contained a considerable proportion of fractionals for a mutation or small deficiency. All these fractionals belonged to type (*b*), *i.e.* all of them carried a normal, non-mutated paternal X in that part of the body which did not exhibit the abnormality. The only kind of type (*b*) fractionals which was never recorded was the triplo-X fractional ($II_1$, *b*); but in view of the difficulty of detecting a mutation in triplo-X tissue, this was to be expected. On the other hand, both complete and fractional aberrants for a dominant mutation (usually small deficiency) on an autosome will very often be viable and were indeed observed frequently. In the following, mosaicism for sex-linked and autosomal mutations or small deficiencies will be treated together.

The most striking fact about the chemically produced fractionals of this type is the high frequency with which they occur in all experiments. After X-ray treatment of ♂♂ the ratio of fractionals to all mutants obtained is usually less than 1 : 6. Thus Patterson (1933) found it to be 1 : 7, Timoféeff-Ressovsky (1937), 1 : 12. Only Moore (1934) obtained as many as 50 per cent. fractionals among his mutants. However, his data are in more than one respect so much in contrast with those obtained by all other workers in the field, including some experiments presented in this paper (Tables IV and V), that it would seem as though either his stocks or his criteria for scoring fractionals were exceptional.

In the progeny of chemically treated ♂♂ fractionals so far have never formed less, and usually considerably more, than one-third of all mutants. As an example, Table II presents an analysis of the aberrations obtained in an experiment in which 2750 sons of treated fathers and untreated attached-X mothers were carefully examined for visible abnormalities.

TABLE II.—FREQUENCY AND TYPES OF TOTAL AND FRACTIONAL VISIBLE ABERRATIONS IN THE PROGENY OF CHEMICALLY TREATED WILD-TYPE ♂♂ AND ATTACHED-X ♀♀

| Type of Aberration | Complete | Fractional | Undecided |
|---|---|---|---|
| (1) Autosomal dominant . . . . . | 22 | 14 | 5 |
| (2) Sex-linked . . . . . | 5 | 1 | 5 |
| (3) Probably genetic, but no progeny obtained . . | 10 | 9 | 4 |
| (4) Probably genetic, gonads not involved . . . | .. | 4 | .. |
| | 37 | 28 | 14 |

*Note.*—The genetical nature of the aberrations recorded in rows (1) and (2) was confirmed by breeding tests. Rows (3) and (4) contain aberrants which for different reasons could not be progeny tested, but in which the genetical nature of the abnormality could be inferred from the phenotype (*e.g.* lozenge eyes). The column headed "undecided" contains cases in which it was not clear whether phenotypical mosaicism had been caused by genetical mosaicism or by asymmetrical expression of a gene present in the whole fly (*e.g.* one wing with plexate venation).

If the undecided cases are not included, the proportion which fractionals form of all aberrants is 28 out of 65 = 43 per cent. Even if all undecided cases are scored as totals, the proportion remains still as high as 28 out of 79 = 35 per cent.

The decision as to whether a mutant is to be considered as complete or fractional is most easily made for mutations of either body colour or bristle shape or colour. Table III brings together data from a number of experiments in which only bristle aberrations (mainly Minutes) were scored for complete or fractional manifestation.

TABLE III.—FREQUENCY OF COMPLETE AND FRACTIONAL ABERRANTS FOR BRISTLE SIZE IN THE PROGENY OF CHEMICALLY TREATED ♂♂ OBTAINED IN SIX DIFFERENT EXPERIMENTS

| Genotype of treated ♂♂ | Genotype of ♀♀ | Approx. No. of $F_1$ | Aberrants | | | Fractionals as per cent. of Total |
|---|---|---|---|---|---|---|
| | | | Complete | Fractional | Total | |
| *B* | *y w sn* | 3700 | 5 | 15 | 20 | 75 |
| *B* | *y w sn* | 3000 | 12 | 12 | 24 | 50 |
| *B* | *y w sn* | 8400 | 78 | 58 | 136 | 43 |
| *Fo4* | *y v f* | 6600 | 26 | 37 | 63 | 59 |
| *Fo4* | *y v f* | 5000 | 21 | 17 | 38 | 45 |
| *Fo4* | *y w sn* | 700 (♀♀ only) | 3 | 14 | 17 | 83 |
| | | | 145 | 153 | 298 | 51 |

In order to meet the objection that special conditions in the genetical make-up of the stocks used might favour the occurrence of mosaics, two experiments with X-rays were carried out on the same *Fo4* stock which had been used for three of the experiments recorded in Table III. In the first experiment, in which *Fo4* ♂♂ were treated with a dose of about 3000 *r*, the $F_1$ contained 44 complete and 6 fractional aberrants for bristle size, a ratio which agrees well with that found by other X-ray workers in their stocks. Even more convincing were the data contained in the second experiment in which two samples of *Fo4* ♂♂ from the same bottles were exposed on successive days, the one to about 2000 *r* of X-rays, the other to chemical treatment producing the same percentage of sex-linked lethals as the irradiation. Again only mutations of bristle size (mainly of the Minute type) were scored. The results are summarized in Table IV.

TABLE IV.—FREQUENCY OF COMPLETE AND FRACTIONAL ABERRANTS FOR BRISTLE SIZE IN THE $F_1$ OF ATTACHED-X ♀♀ AND TWO GROUPS OF *Fo4* ♂♂, ONE OF WHICH HAD BEEN IRRADIATED WITH 2000 *r*, WHILE THE OTHER HAD BEEN GIVEN CHEMICAL TREATMENT PRODUCING THE SAME PERCENTAGE OF SEX-LINKED LETHALS (7 PER CENT.)

| | X-rays | Chemical Treatment |
|---|---|---|
| No. of $F_1$ ♂♂ examined | 1946 | 2245 |
| Complete aberrants | 9 | 10 |
| Fractional aberrants | 0 | 8 |
| No. of $F_1$ ♀♀ examined | 2215 | 2592 |
| Complete aberrants | 11 | 11 |
| Fractional aberrants | 1 | 9 |
| Total No. of $F_1$ flies examined | 4161 | 4837 |
| Complete aberrants | 20 | 21 |
| Fractional aberrants | 1 | 17 |
| Total No. of aberrants | 21 | 38 |
| Fractionals as per cent. of total No. | 5 per cent. | 45 per cent. |

The difference between the results in the two series is obvious, and statistically highly significant. Thus the high proportion of fractionals obtained after chemical treatment appears to be due not to some property inherent in the stocks used, but to the special way in which chemical treatment as opposed to irradiation exercises its mutagenic effect.

In a number of experiments (some of them included in Table III) treated ♂♂ were mated to *y w sn* ♀♀. This allows of the detection in $F_1$ of small deficiencies for or mutations to one of the marker genes used. Naturally, such specific effects are rather rare, but the limited number of aberrants obtained again shows a high proportion of fractionals. This is seen from Table V, which also brings the result of a parallel test with X-rays.

TABLE V.—FREQUENCY OF COMPLETE AND FRACTIONAL SMALL DEFICIENCIES FOR OR MUTATIONS TO ONE OF THE THREE MARKER GENES IN THE PROGENY OF CHEMICALLY TREATED OR IRRADIATED ♂♂ AND *y w sn* ♀♀

| Genotype of ♂♂ | Treatment | Dose (1) | Approx. No. of Wild-type Daughters | No. of ♀♀ Exhibiting one of the Markers | |
|---|---|---|---|---|---|
| | | | | Complete | Fractional |
| $sc^{S_1}$ (2) | Chemical | 8 per cent. lethals | 5,600 | 3 | 3 |
| *Fo4* | ,, | 13 ,, ,, | 700 | 1 | 1 |
| *Fo4* | ,, | not determined | 5,000 | 0 | 1 |
| *B* | ,, | ,, | 4,000 | 8 | 1 |
| All chemical tests combined | | | 15,300 | 12 | 6 |
| *Fo4* | X-rays | approx. 3000 *r* | 3,600 | 6 | 0 |

*Note.*—(1) Owing to the lack of a suitable non-biological scale of comparison between doses of irradiation and chemical treatment, the chemical dose was determined by the rate of sex-linked lethals obtained in a parallel C/B test with a sample of the treated ♂♂.

(2) On account of the eversporting for yellow found in $sc^{S_1}$ heterozygotes, the *y* gene was disregarded in the first experiment.

Thus, in three out of the four tests with chemical treatment, fractionals formed at least 50 per cent. of all aberrants, and the combined proportion for all tests is still as high as

33 per cent. These data, although small, are in good agreement with those obtained for mutations of bristle size.

To what extent gene mutations proper participate in this tendency to mosaicism in $F_1$ individuals is difficult to assess, on account of the impossibility of distinguishing between a mutation and a small deficiency. Most of the Minutes in Tables III and IV are presumably small deficiencies rather than mutations, and this applies even more to aberrations by which marker genes in heterozygous ♀♀ are uncovered (Table V). The greatest claim to be classified as gene mutations proper have on the one hand those aberrations which, although sex-linked, are viable in ♂♂, and on the other hand members of a series of multiple allelomorphs. A number of fractionals for aberrations which satisfy one of these conditions have been collected in the course of these experiments. They include one allelomorph of forked and one of singed, both viable in ♂♂; two different shades of lozenge, one of them twice; and three of the higher members of the white series, two of them in the same ♂. This ♂ was the son of a chemically treated father and of an attached-X mother. His left eye was buff-coloured except for one very small apricot-coloured sector, whilst the right eye was apricot-coloured except for one small buff sector. Breeding tests revealed that his gonads, too, were mosaic for these two colour factors, both of which were allelomorphs of white, and non-lethal to ♂♂ hemizygous for them.

### *D. Comparison of the Different Types of Fractionals*

In Table VI the frequencies of the various types of fractionals have been entered into an abbreviated scheme of fig. 1.

TABLE VI.—FREQUENCIES OF VARIOUS TYPES OF CHEMICALLY PRODUCED FRACTIONALS IN THE $F_1$ OF TREATED ♂♂ (FOR SCHEME OF TABLE COMPARE FIG. 1)

| Paternal X . . | A, Normal | B, with Large Deletion | | C, with Small Deficiency or Mutation |
|---|---|---|---|---|
| Maternal X's . . | Free | Free | Attached | Free or attached |
| Complete aberrants . | .. | 9 | 47 | Many hundreds |
| Fractionals type (*a*) (chromosome loss). | Gynandromorphs, apparently not more frequent than after irradiation | 0 | 0 | 0 |
| Fractionals type (*b*) (restitution). | .. | 2 | 3 | About 50 per cent. |

It will be seen from Table VI that all fractional aberrants (columns B and C in the table) obtained in the progeny of chemically treated ♂♂ belonged to type (*b*), *i.e.* had a complete and normal paternal X-chromosome in the non-aberrant portion of their body. In the only class which yielded sufficient data for statistical evaluation, that of aberrants for mutations or small deficiencies (C), fractionals form a very significantly larger proportion of all aberrants than in the progeny of irradiated ♂♂. The number of aberrants for large deletions (B) was too small to allow of valid conclusions about their frequency; but the fact that 5 out of 61 aberrants of this type appeared as fractionals makes it appear that in this class, too, fractionals are not infrequent, although possibly less frequent than in Class C. No comparable data on fractionals for large deletions in the $F_1$ of irradiated ♂♂ could be found in the literature.

On the other hand, the data give no evidence for an increased frequency of fractionals due to chromosome loss (type (*a*)) after chemical treatment as compared with irradiation.

### 3. Fractionals in Successive Broods

In a few experiments (some of them included in previous tables) the treated ♂♂ were mated to a succession of fresh virgin ♀♀ at regular intervals after treatment, and the broods produced by each group of ♀♀ were recorded separately. Table VII shows the data from these experiments as far as they relate to the incidence of complete and fractional aberrants for bristle size (mainly of the Minute type). The first part of the table contains combined data from two experiments in which new ♀♀ were given every third day; the second, combined data from two experiments with changes of ♀♀ at 5-day intervals.

Table VII.—Proportion of Complete and Fractional Aberrants for Bristle Size in Successive Broods from the same Chemically Treated ♂♂ and Fresh Virgin ♀♀

| Brood | Time in Days between Treatment of ♂♂ and Mating with ♀♀ | Aberrants for Bristle Size in $F_1$ | | | |
|---|---|---|---|---|---|
| | | Complete | Fractional | Total | Fractionals as per cent. of Total |
| 1 | 0–3 | 6 | 23 | 29 | 89 |
| 2 | 3–6 | 12 | 14 | 26 | 54 |
| 3 | 6–9 | 11 | 17 | 28 | 61 |
| 4+ | 9+ | 6 | 2 | 8 | 25 |
| 1 | 0–5 | 63 | 49 | 112 | 43 |
| 2 | 5–10 | 17 | 17 | 34 | 50 |
| 3+ | 10+ | 12 | 5 | 17 | 29 |

Both series agree in showing that the high frequency of fractionals, characteristic for chemical treatment, is found up to the 9th or 10th day after treatment. Following that, there seems to be a decline in the percentage of fractionals, which in the first part of the table reaches the borderline of significance (P between ·05 and ·02). In any case, there can be no doubt that fractionals appear even in the latest broods. In broods produced by ♀♀ which had been mated to the treated ♂♂ 12 days or later after treatment (in Table VII included in the 9+ and 10+ groups), 3 out of 7 bristle aberrations appeared in fractional individuals.

## Discussion

In the first part of this paper it has been shown that chemical treatment of embryos results in mosaic eye-spots which on the average are smaller than in flies which at a comparable age have been given X-ray treatment. This would be difficult to understand if chemical substances, like X-rays, exercised their influence on the chromosomes at the time of treatment itself. It becomes understandable if for chemical treatment we assume a time-lag between treatment and effect. During a period of a few hours only, an embryonic cell, precursor of, say, 100 facets, into which one or several molecules of a mutagenic substance have been introduced, will have divided into a number of smaller cells, each of them precursor of a much smaller number of facets. If then the chance of effective molecules coming in contact with the chromosomes is only small in each cell, it is likely that this chance will usually become realized in not more than one of the descendant cells, and thus will give rise to a much smaller spot than if the treatment had been effective at once in the original cell, as assumed for X-rays.

A time-lag between treatment and effect may arise in several ways. The mutagenic molecules may require some hours before they have penetrated through the nuclear membrane and reached the chromosomes. Or the final mutagenic molecule may be a derivative of a long chain of reactions which in the colloidal system of the cell requires several hours for its completion. Or the production of this final mutagenic molecule may depend on the presence

in the cell of some metabolic product which is absent in young embryonic cells and only gradually accumulates as development proceeds. A further possibility will be mentioned below.

To account for the high frequency of fractionals in the progeny of chemically treated ♂♂ requires much more than the assumption of a time-lag of a few hours. It has been shown in Table VII that the percentage of fractionals remains sensibly the same in broods produced up to 9 or 10 days after treatment, and that even in broods produced 12 days or more after treatment fractionals still occur. It does not seem conceivable that a chemical reaction which, as shown by the presence of complete mutants already in the very first broods, can be completed in a short time should in a certain proportion of the cases require 10 or 12 days for its completion. It might, however, be assumed that mutagenic molecules, although present in the treated cell at the time of treatment or shortly afterwards, may linger on for a long time without a chance of effectively contacting a chromosome. This assumption has been rendered unlikely by yet unpublished experiments in which the rate of lethal mutations was not increased in untreated sperm which was introduced into treated ♀♀; thus at the latest a few days after treatment the cytoplasm no longer seems to contain mutagenic molecules capable of inducing lethals in the chromosomes.

It seems more likely that the difference between the frequencies of fractionals after irradiation and chemical treatment is due to some modification, through the chemical treatment, of the mechanism which causes fractionals after irradiation. Two theories concerning this mechanism have been put forward. Muller (1940) assumes that all chromosomes in the mature sperm are undivided and in this condition are broken by the impinging radiation. Reunion of broken ends, however, is deferred till after fertilization, when the chromosomes divide. Fractionals arise when of the two broken chromatids in the male pronucleus one undergoes "restitution" while the other enters into a new type of arrangement. According to this theory, the ratio of fractional to complete mutations depends on the timing relationship between prophase splitting on the one hand and reunion of broken ends on the other. A change in this timing relationship after chemical treatment in the direction of speeded-up prophase splitting or of delayed reunion of broken ends would account for the excess of fractionals. If this is the explanation, then the proportion of X-ray fractionals might be raised by subsequent chemical treatment of the irradiated sperm.

As Muller pointed out himself, his theory of mosaicism meets with difficulties in the interpretation of fractionals for what appear to be gene mutations proper, not due to breakage and reunion. Especially difficult to explain on his assumption is the case of the mosaic ♂, son of a treated father and an attached-X mother, who both in this phenotype and gonads was mosaic for two different allelomorphs of white. It should be noted that two similar cases have been reported in X-ray literature. Panshin (1935) describes a case in which a son of an irradiated father was both somatically and gonadically a mosaic for two shades of lozenge. Neuhaus (1935) mentions a ♂, son of an irradiated father, who carried what appeared to be two different allelomorphs of yellow, although he bred for only one of them.

Muller's interpretation of X-ray fractionals was offered as an alternative to that previously proposed by Patterson (1933), who attributed mosaicism in the $F_1$ to the presence in the sperm of a certain proportion of already split chromosomes. Since the proportion of spermatozoa which at the time of treatment are already separated into chromatids must be independent of the subsequent treatment, the difference in the proportions of fractionals after irradiation and chemical treatment provides a strong argument against Patterson's theory in its original form. It may, however, be modified so as to assume that all or many of the chromosomes in mature spermatozoa, although not yet effectively split into chromatids, are already internally duplicated, and that one action of the mutagenic substance consists in an attack on the link between the two potential daughter genes, thus leading to an increase of effectively split chromosomes or parts of chromosomes. If this were true, one might expect chemical treatment to increase the proportion of fractionals produced by subsequent irradiation of ♂♂. One such experiment is under way. As a preliminary step to further tests of this kind it was possible to establish that the frequencies of sex-linked lethals after the two treatments are purely additive. Alternatively it may be assumed that the chemical reaction between a mutagenic molecule and an already internally duplicated chromosome has only a limited

chance of equally affecting both homologous loci. Failure to do so will result in a fractional for mutated and unmutated tissue. In rare cases the effect may be transmitted to the adjoining homologous locus in a slightly modified form, and this would result in a mosaic for two different allelomorphs, like the one described above. In point of fact, this is the way in which Panshin has tried to interpret the similar mosaic obtained in his X-ray experiment.

There exists, however, a further difficulty for the interpretation of chemically produced fractionals. X-ray experiments on the frequencies of recessive lethals in successive broods (Hanson and Heys, 1929; Harris, 1929) have been taken to indicate that with repeated re-matings of ♂♂ the mature sperm present at the time of treatment is exhausted after about 12 days, and that subsequent broods are produced by sperm which at the time of treatment was still in the meiotic or pre-meiotic stage. This was confirmed by Harris's observation that in all broods produced at least 12 days after treatment the induced lethals tended to occur in bunches of identical mutations. Similarly, in unpublished experiments with chemical mutagens, a significant change in the rate of induced lethals was recorded 10 days after treatment when the ♀♀ mated to the treated males were changed every fifth day, and as early as 6 days after treatment when the change was carried out every third day. Demerec and Kaufmann (1941), working with X-ray induced dominant lethals, came to the conclusion that it takes 19 days for the mature sperm present at the time of treatment to be exhausted; but in their experiments the treated ♂♂ were only allowed to mate at intervals of several days and kept without ♀♀ in the intervening periods, which may have delayed the time required for complete exhaustion of the originally available sperm. Pontecorvo (1944), arguing from cytological observations on synchronization of divisions in the germ track, believes that all estimates of the time required for exhaustion of mature sperm which are based on a change in the rate of induced lethals tend to give too high values, and that the actual period during which post-meiotic sperm is available in a repeatedly mated ♂ does not exceed 10 days. If this is correct, it must be assumed that mutated chromosomes which appear in broods produced 10–12 or more days after treatment have taken part in one or more cell divisions between treatment and entrance into the fertilized ovum. Since after chemical treatment even these late broods still contain fractionals, it would appear as though in these cases mosaicism originated at a later division than the one following treatment. This cannot be explained by a modification of either Muller's or Patterson's scheme. It suggests a third possibility for the origin of fractionals: it seems conceivable that the primary action of a mutagenic substance on a chromosome consists in the creation of an instability at the affected locus, perhaps through the formation of a loose compound between chromosome and mutagenic molecule, and that in a certain proportion of cases this instability may be carried over one or more cell divisions before it finally leads to a breakdown at the affected locus. This mechanism could also be drawn upon to account for the small size of mosaic spots in flies which have been treated as embryos, for which other explanations have been discussed above.

If, indeed, this type of after-effect were possible with chemical treatment, one would expect that, especially after treatment of mature sperm, fractionals should sometimes arise at cleavage divisions later than the first. This should show up in the size of the mutated area in the resulting fly, and should lead to a tendency for these areas to comprise less than one-half, or even less than one-quarter, of the body surface. As a matter of fact, it was found that of 75 chemically produced fractionals in which the mutated area had been mapped according to Patterson's and Stone's scheme for gynandromorphs (1938), 22 had less than one-quarter of the body surface made up of mutated tissue. This seems a smaller average size than is usually recorded for $F_1$ fractionals which have been produced by irradiation; but in view of the probability that different workers may have used different criteria for classification, and in view of the indeterminate cleavage pattern of *Drosophila* (Parks, 1936), not much weight can be given to this observation.

Large-scale experiments to solve the question of an after-effect of chemical treatment by exposing larvæ gave only doubtful results, owing to the fact that the sensitivity of larvæ to these chemicals is such that the majority were killed by treatment of only slight mutagenic efficiency. A study of the $F_1$ of chemically treated ♀♀ appears more promising. It is well known that the chromosomes in the unfertilized egg are still undergoing the first meiotic division and have to carry out the second before taking part in cleavage. Fractionals obtained

in the $F_1$ of treated ♀♀ would therefore provide convincing proof of the possibility of an after-effect of chemical treatment on chromosomes. Experiments of this nature are under way, and a few fractional offspring of treated mothers have already been obtained; but since these were mosaics for autosomal aberrations, the possibility cannot be excluded that they arose from spermatozoa which had been introduced into the ♀ at a time when the genital tracts still contained sufficient active substance to affect the paternal chromosomes.

If further experiments should substantiate the existence of an after-effect of chemical treatment, it might be worth while to re-examine radiation literature from this point of view. Stadler (1941) has made a good case for an after-effect of ultra-violet radiation, but the delay observed in his experiments did not exceed one cell division. Delayed action of X-rays has been claimed repeatedly in the past (*e.g.* Neuhaus, 1935); but on the whole the great mass of the evidence has not supported this contention. Recently Bishop (1942), using cytological methods on irradiated grasshopper embryos, has repeated the claim. It should also be noted that the differences between the effects of chemical treatment and irradiation which have been described in this paper are only quantitative, and that even for the outstanding occurrence of a fractional for two different allelomorphs at the same locus there exist two parallels in X-ray literature. If re-opening this question not only for chemical mutagens, but also for various types of radiation, should show that after-effects may occur with all these treatments although very rarely with X-rays, this would lend support to the school of thought which attributes the mutagenic properties of X-rays to indirect action via short-chain chemical reactions.

Finally, mention should be made of the observation that mosaicism due to loss of a whole or deleted chromosome does not appear to be increased in frequency after chemical as compared with radiation treatment. If systematic tests for the frequencies of gynandromorphs after either kind of treatment should bear out this impression, it might shed some new light on the relationship between chromosome loss and re-arrangement which has recently received attention in connection with the problem of dominant lethality (Pontecorvo, 1942; Demerec and Fano, 1944).

## Summary

Mustard gas and related substances have been used for producing mosaics in *Drosophila melanogaster*. When mosaicism is produced by treating embryonic stages, the mosaic spots tend to be smaller than after irradiation of similar stages. In the $F_1$ of chemically treated ♂♂ mosaics form at least one-third, usually more, of all aberrants. This is a significantly higher proportion than is found in the progeny of irradiated ♂♂. Gynandromorphs, on the other hand, do not seem to be more frequent after chemical treatment than after irradiation of ♂♂. The frequency of fractionals remains sensibly the same in successive broods produced by repeatedly changed ♀♀ up to the ninth and tenth day following treatment, and even in broods produced 12 days or more after treatment, fractionals still formed an appreciable proportion. Of the various possible interpretations of these results, the assumption of an after-effect of chemical treatment on the chromosomes is best fitted to cover all observations; but more direct proof is needed before it can be accepted.

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# THE INDUCTION BY MUSTARD GAS OF CHROMOSOMAL INSTABILITIES IN DROSOPHILA MELANOGASTER

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The induction by mustard gas of chromosomal instabilities in Drosophila melanogaster.

1946

The discovery (Auerbach 1943; ~~in press~~; Auerbach and Robson, 1946; in press) that mustard gas is comparable to X-rays and similar physical agencies in its ability to produce mutations and chromosome re-arrangements has opened up a new line of approach to the problem of gene mutation. It is to be expected that a comparative study of the mechanism by which chemical substances on the one hand and physical agencies on the other exercise their mutagenic effects will further our understanding of the process of mutation itself. One of the first questions to be tackled in the early days of radiation genetics was the possibility of a delayed mutagenic action of irradiation (Muller 1927, Timoféeff-Ressovsky, 1930, 1931, Grüneberg 1931). The bulk of the evidence (see however Bishop 1942) indicates that X-ray induced mutations and chromosome breaks arise as an immediate effect of the irradiation, although after treatment of mature spermatozoa new recombinations of broken chromosomes may be delayed until the spermatozoon has entered the egg. Data obtained by Stadler (1939) suggest that after ultraviolet radiation of pollen grains the mutational process often is not completed before the treated chromosome has split into its two daughter chromatids. This results in a high proportion of mosaics. A similarly high proportion of mosaics has been found in the progeny of Drosophila ♂♂ which had been treated with mustard gas (Auerbach and Robson 1946, Auerbach 1946). This raises the question of a possible delayed action of the chemical mutagenic treatment.

Two series of experiments were carried out to investigate this point. The first consisted in a study of the frequency of sex-linked lethals in the $F_3$ from treated ♂♂; its object was to detect mutations which had arisen, not in the

treated/-

treated chromosome itself, but in chromosomes descended from it. The second series of experiments consisted in an analysis of apparent "semi-lethals" among the progeny of treated ♂♂; its object was to discover how many of these semi-lethals were only apparent and caused by the presence of a mutated patch in the gonads of an $F_1$ individual. Both methods agreed in revealing the frequent occurrence, among the $F_1$ of treated ♂♂, of individuals in which part of the gonad carried a mutated gene, and the remaining part its normal allelomorph. Mosaics of this type (see Fig.2,a) will be called "gonadic mosaics" as distinct from "gonosomic mosaics" (Sidky 1940), in which the whole of the gonad carries a mutated gene while all or part of the soma carries its normal allelomorph (Fig.2.c). When the progeny of chemically produced gonadic mosaics was again tested by breeding, it was found that in several instances some of the daughters of a gonadic mosaic in their turn were gonadic mosaics for the same mutation. These findings are taken to suggest a delayed action of the treatment in the sense that it may create instabilities at certain loci, i.e. a tendency in these loci to repeated identical mutation.

Most of the experiments have been carried out already in 1941, 1942, and 1943, but, owing to a security ban, publication had to be deferred until now.

## Frequency of lethals in $F_3$

With a scheme of crosses like the classical ClB method, or any of its more recent modifications, sex-linked lethals which arise in the germ cells of the $P_1$ ♂♂ are detected by the absence of ♂♂ with the treated X-chromosome in the $F_2$. Similarly, sex-linked lethals which, after treatment of the $P_1$, arise in the germ track of $F_1$ ♂♂ or ♀♀ are detected by the absence of ♂♂ with the treated X-chromosome in $F_3$. Therefore, if care is taken that no lethals are lost from the treated chromosome by crossing-over, an excess of lethals in $F_3$ over the controls/-

controls may be taken to indicate a delayed effect of the treatment. Tests of this kind have been carried out for irradiated material by Muller (1927), Timoféeff-Ressovsky (1930, 1931), and Grüneberg (1931). They mated X-rayed ♂♂ to attached-X ♀♀; the $F_1$ ♂♂, which of course could not carry a sex-linked lethal on their X-chromosome, were subjected to a ClB-test. In no instance was a significant difference between experimental series and controls obtained, and this was taken to indicate the absence of an after-effect of the treatment. When this type of cross was used on ♂♂ which had been exposed to mustard gas vapours (for technique see Auerbach and Robson, in press) the $F_3$ of the treated series yielded a slight excess of lethals over the controls, but the difference was not significant.

The negative or inconclusive results obtained with this method are, however, open to the objection that sex-linked lethals, especially small deficiencies which arise in the germ track of the hemizygous ♂, may prevent the affected cells from developing into mature spermatozoa. This objection holds to a much lesser extent for the ♀ in which the deleterious effect of a lethal on one X-chromosome will in most cases be covered up by the presence of the normal allelomorph in the other. Therefore, tests were carried out in which attention was directed to the rate at which mutations arise in the germ track of daughters of treated ♂♂. The general mating scheme is given in Fig.1, which also shows how the test can be extended to further generations. This method has already been used by Muller (1927) with irradiated flies. He obtained a slight excess of lethals in the $F_3$ of the treated series, and he considered the possibility that $F_1$ ♀♀ may be mosaic for a sex-linked lethal, similar to the fractionals for a visible mutation.

Three experiments were carried out in accordance with the general scheme of Fig. 1. The detailed mating schemes were slightly different in the three

tests; but since in their essential features they were similar, it is sufficient to show one of them in detail.

Genetical details of one of the mating schemes whose general nature is illustrated in Fig.1.

$P_1$ ♂♂ $sc^{Sl}$(InS)$w^a sc^8$(treated) x ♀♀ ClB/scar mass matings.

$F_1$ ♀ $sc^{Sl}$(Ins)$w^a sc^8$/ClB x brother scar pair matings.

$F_2$ (X) ♀ $sc^{Sl}$(Ins)$w^a sc^8$/scar x ♂ wild-type pair matings.

$F_3$ Absence of ♂♂ with $w^a$ eyes is due to a sex-linked lethal which arose in the germ track of the $F_1$ ♀.

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(X) Chosen from $F_2$ cultures which contained $w^a$ ♂♂.

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The data obtained in three experiments of this type are summarized in Table 1.

Table 1.

Frequency of sex-linked lethals in the $F_3$ of chemically treated ♂♂. The lethals occurred first in the germ track of daughters of the treated ♂♂.

| Experiment | % of sex-linked lethals in $F_2$(X) | No.of tested chromosomes | Lethals and semi-lethals in $F_3$ | | | |
|---|---|---|---|---|---|---|
| | | | Lethals | | No. of semi-lethals. | % of lethals + semi-lethals |
| | | | No. | % | | |
| I | 9 | 1161 | 2 | .2 | 3(+) | .4 |
| Control to I | ---- | 1165 | 0 | 0 | 0 | 0 |
| II | 13 | 828 | 21 | 2.5 | 7 | 3.4 |
| Control to II | ----- | 1057 | 3 | .3 | 1 | .4 |
| III | 10.1 | 1049 | 45 | 4.5 | not scored | |
| Total treated | | 3038 | 68 | 2.2 | | |
| Total controls | | 2222 | 3 | .13 | | |

(X) This percentage is used to measure the dose of mustard gas which reached the gonads of the treated ♂♂.

(+) All of them almost completely lethal.

It will be seen that the frequency of lethals in the $F_3$ of treated flies was considerably and significantly in excess of that obtained in the controls; moreover it exceeded markedly the normal range of spontaneous mutability observed in our laboratory stocks of Drosophila melanogaster. The same applies to Experiments II and III taken individually. Only in Experiment I, in which the dose of mustard gas was lowest, the difference between treated series and controls, although in the right direction, did not reach the level of statistical significance (P for lethals and semi-lethals combined between .05 and .1)(x)

Experiment III was carried on for one more generation (see Fig.1) so as to obtain the frequency of lethals in $F_4$. These lethals would have arisen in the germ track of $F_2$ ♀♀. No lethal was obtained in 1005 tested chromosomes.

It has been said that the lethals observed in $F_3$ must have arisen in the germ track of $F_1$ ♀♀. It was of interest to know how many $F_1$ ♀♀ shared in the production of these lethals. It was conceivable that all lethals were identical and derived from one gonosomic or gonadic mosaic in $F_1$; on the other hand, each lethal might have arisen singly - and therefore presumably late in development - in a different $F_1$ ♀. These are the two extremes between which the actual distribution of the lethals would be expected to lie. In order to decide this point, which gives an indication of the time at which the lethals arose in $F_1$, Experiment III was carried through in such a way that every lethal could be traced back to a particular $F_1$ ♀. Altogether 77 $F_1$ ♀♀ were used for producing the $F_2$. The 1049 $F_2$ ♀♀ were kept in separate groups according to their mother. The distribution of the 45 lethals on these groups showed that all of them occurred among the progeny of 3 out of the 77 mothers. One of these 3 $F_1$ ♀♀ produced only 1 daughter heterozygous for a lethal among 12 tested daughters; this may conceivably have been

(x) The estimate for P has been based on a contingency table with Yates' correction for small numbers. The author is indebted to Dr O.Kermack for pointing out the advantage of this formula for mutation work.

due to spontaneous mutation. The second ♀ had 33 tested daughters with red eyes (i.e. carrying the treated chromosome). 29 of these were heterozygous for a lethal, the remaining 4 carried a normal wild-type chromosome. The third ♀ had 18 tested daughters with the treated chromosome, 15 of which were heterozygous for a lethal, while 3 carried no lethal on the treated X. In addition, this last ♀ also had one red-eyed daughter which appeared to carry a semi-lethal because she produced only a few wild-type sons among a large progeny. This case turned out to be of special interest, and will be dealt with later. On the whole, then, two, possibly three, among 77 tested daughters of treated ♂♂ were gonadic mosaics for a lethal on the treated X-chromosome.

Gonadic mosaics are rare in the progeny of X-rayed ♂♂. It therefore seemed desirable to compare their relative frequencies after irradiation and mustard gas treatment. To this purpose an analysis of apparent "semi-lethals" produced by the two types of treatment was undertaken.

Gonadic mosaicism for a lethal as a basis for spurious "semi-lethality".

In the $F_2$ of a ClB or equivalent test carried out on ♂♂ which have been treated with an effective mutagen there usually occurs a certain proportion of cultures in which ♂♂ with the treated X-chromosome, although not completely absent as in the case of a lethal, yet form a strikingly low proportion. The usual interpretation of this type of culture is that the $F_1$ ♀ producing it carried on the treated X-chromosome a recessive mutation which severely impairs the viability of the ♂♂ so that only a small proportion of these reach the imaginal stage. A mutation of this type has been called a "semi-lethal" in contrast to the "full" lethal which impairs viability of the ♂♂ to such an extent that practically none reach the imaginal stage. There is no doubt that in very many cases semi-lethals are responsible for a low proportion of ♂♂ of a particular type in a culture (see

particularly Timofeéff-Ressovsky 1935). However, the same type of culture would also be produced if the $F_1$ ♀, instead of being heterozygous for a semi-lethal, were heterozygous for a full lethal in part of her ovary and homozygous for its normal allelomorph in the remainder, i.e. if she were a gonadic mosaic for a full lethal. A decision between these two alternatives can be made by breeding tests. A ♀ which is heterozygous for a semi-lethal will transmit this gene to all her progeny which receive the treated X-chromosome. Her sons, when mated to attached-X ♀♀, will yield a progeny with low sex-ratio, and her daughters will have few sons with the treated X. If, on the other hand, the $F_1$ ♀ is a gonadic mosaic for a full lethal, her surviving sons are those which receive their X-chromosome from the non-mutated portion of the ovary and thus will yield progenies with normal sex-ratios when mated to attached-X ♀♀. Her daughters will be of two types: those heterozygous for a lethal, and those with no lethal on the treated X-chromosome.

Table 2 gives the breeding record of an $F_1$ ♀ which appeared to be heterozygous for a semi-lethal sexlinked mutation, but which in reality was a gonadic mosaic for a full lethal.

Table 2

Analysis of a semi-lethal effect in $F_2$ caused by gonadic mosaicism for a lethal in $F_1$.

$P_1$ treated ♂ Fo4 x ClB/scar

$F_1$ ♀ ClB/ + (treated) x ♂ $sc^{Sl}$(InS)$w^a sc^8$

$F_2$ 85 ♀♀, 9 ♂♂.

Test matings with $F_2$

(a) 3 ♂♂ mated individually to XX ♀♀ gave 1:1 sex-ratios in $F_3$.

(b) 18 ♀♀ $sc^{Sl}$(InS)$w^a sc^8$/ + mated individually gave the following progenies:

| ♀ | Number of daughters | sons $\underline{w^a}$ | sons + | Treated X | |
|---|---|---|---|---|---|
| 1 | 33 | 10 | 0 | | with lethal |
| 2 | 31 | 10 | 11 | normal | |
| 3 | 39 | 17 | 0 | | with lethal |
| 4 | 27 | 9 | 17 | normal | |
| 5 | 26 | 11 | 0 | | with lethal |
| 6 | 50 | 15 | 0 | | with lethal |
| 7 | 24 | 6 | 0 | | with lethal |
| 8 | 22 | 6 | 7 | normal | |
| 9 | 26 | 8 | 12 | normal | |
| 10 | 36 | 19 | 16 | normal | |
| 11 | 38 | 16 | 0 | | with lethal |
| 12 | 37 | 7 | 0 | | with lethal |
| 13 | 35 | 6 | 11 | normal | |
| 14 | 46 | 25 | 27 | normal | |
| 15 | 28 | 10 | 7 | normal | |
| 16 | 41 | 9 | 0 | | with lethal |
| 17 | 42 | 11 | 0 | | with lethal |
| 18 | 39 | 13 | 0 | | with lethal |
| $F_3$ | All the lethals were confirmed in mass-cultures. | | | 8 normal | 10 with lethal |

Altogether 35 analyses of this kind were carried out, 15 on semi-lethals produced by X-rays, and 20 on semi-lethals produced by mustard gas. The data are summarized in Table 3.

Table 3

Analysis of apparent semi-lethals.

| | No. Tested | Viability mutations. | Gonadic mosaics for a lethal |
|---|---|---|---|
| X-rays | 15 | 14 | 1 |
| Mustard gas | 20 | 11 | 9 |

Whereas about half of the apparent semi-lethals in the chemical series are due to gonadic mosaicism, only one such case was detected among 15 apparent semi-lethals in the X-ray series. If, as these data indicate, gonadic mosaics are frequent after chemical treatment, but infrequent after X-radiation of the father, they/-

they should tend to swell the number of apparent semi-lethals in mustard gas tests as compared with X-ray experiments. A valid comparison of this kind would require recording of the sex-ratio in every $F_2$ culture. This has not been done in any of the present experiments. Nevertheless, it seems worth noting that in one experiment, in which ♂♂ from the same culture bottles were exposed either to X-radiation or to mustard gas treatment which produced the same percentage of sex-linked lethals as the irradiation, the ratio of striking semi-lethals to full lethals was higher in the progeny of chemically treated ♂♂, the difference being on the borderline of statistical significance.

The question arises: is this difference in the frequencies of gonadic mosaicism in the progeny of chemically treated and of irradiated ♂♂ simply a consequence of the well established fact (Auerbach and Robson, 1946: Auerbach,1946) that in general mosaicism is very markedly higher after chemical treatment, or is there also a relative excess of gonadic mosaics among mosaics of all types in chemical experiments? In order to decide this point, it is necessary to establish for either type of treatment the ratio between gonadic mosaics on the one hand and mosaics with uniform gonads on the other. For visible mosaics produced by X-radiation of ♂♂ this has been done repeatedly in the past. The data are included in Table 4. When lethal mutations are studied it is not possible to detect mosaics with uniform gonads by observation. As Fig.2 shows they will be scored either as not carrying a lethal at all (type b) or as heterozygous for a lethal (type c). It is, however, possible to estimate their frequency on the basis of two plausible assumptions: (1) that in ♀♀, in which germinal selection against a sex-linked lethal may be presumed to be absent, the two types b and c occur with approximately equal frequency, and (2) that the ratio of complete to mosaic mutants in the $F_1$ of treated ♂♂ is the same for lethals as for visible mutations, i.e. approximately 6:1 after irradiation (Patterson 1933) and approximately 1:1 after chemical treatment (Auerbach 1946).

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If it be assumed that $\underline{l}$ is the number of apparent heterozygotes for a lethal, including mosaics of type c, $2\underline{u}$ the number of mosaics with uniform gonads, and $\underline{m}$ the number of gonadic mosaics of type a, the following simple equations obtain for the two kinds of treatment:

X-rays $$\frac{\underline{l} - \underline{u}}{2\underline{u} + \underline{m}} = 6$$

Mustard gas $$\frac{\underline{l} - \underline{u}}{2\underline{u} + \underline{m}} = 1$$

Where $\underline{l}$ and $\underline{m}$ are known from observation, $2\underline{u}$ can be calculated. A source of error is introduced when not all $F_1$ ♀♀, but only apparent semi-lethals are analysed by breeding tests, because with this sampling method gonadic mosaics in which the mutated portion of the gonad is small will escape detection. Therefore ratios obtained from such samples (called "selected" in Table 4) will tend to give too low an estimate of the true ratios. Especially in the last experiment listed in the table, selection of semi-lethals for analysis had been carried out in such a way that very probably not all gonadic mosaics could be detected.

Table 4

(Ratio of gonadic mosaics to mosaics with uniform gonads)

(In the case of lethals, the frequency of mosaics with uniform gonads has been estimated by calculation - see text).

| | Type of mutation | Mosaics with gonads | | ratio | Sample |
|---|---|---|---|---|---|
| | | mixed A | uniform B | A:B | |
| X-rays (Neuhaus 1935) | visible | 3 | 61 | 1:21 | |
| (Pontecorvo 1940) | | 1 | 13 | 1:13 | |
| | lethal | 1 | 21 | 1:21 | selected (X) |
| Mustard gas | lethal | 2 | 4 | 1:2 | (X) |
| | | 2 | 10 | 1:5 | selected (X) |
| | | 3 | 31 | 1:10 | selected (X) |

(X) Only apparent semi-lethals selected for analysis.

Although the data are not suitable for statistical analysis, they suggest a difference between the ratios of the two kinds of mosaics after irradiation and after chemical treatment. In the progeny of irradiated ♂♂, gonadic mosaics always appear to form markedly less than one tenth of all mosaics. After mustard gas treatment a ratio as low as 1:10 was obtained only in one estimate which for several reasons probably was too low.

## Breeding analysis of the progeny of gonadic mosaics

In general, a ♀ who is a gonadic mosaic for a lethal, has two types of daughters (see Table 2): those without lethal, and those heterozygous for a lethal. In several cases, however, it was observed that a gonadic mosaic in addition to these two expected types produced also one or more daughters which, like their mother, appeared to carry a semi-lethal. One such case has been mentioned in the first part of the paper. When these apparent semi-lethals were analyzed by breeding tests, they were again found to be due to gonadic mosaicism. The frequency with which this occurred can be seen from Table 5.

Table 5/-

Table 5

The frequency of gonadic mosaics among daughters of gonadic mosaics. The mothers were all daughters of chemically treated ♂♂.

| $F_1$ ♀ | No. of daughters examined | Genotypes of daughters ($F_2$) | | | Genotypes of daughters of $F_2$ mosaics ($F_3$) | |
|---|---|---|---|---|---|---|
| | | heter.for lethal | no lethal | gonadic mosaic for lethal. | heter.for lethal. | no lethal |
| A | 33 | 29 | 4 | 0 | | |
| B | 18 | 15 | 2 | 1 | 1 | 1 |
| C | 21 | 17 | 4 | 0 | | |
| D | 9 | 4 | 5 | 0 | | |
| E | 18 | 14 | 3 | 1 | 8 | 2 |
| F | 9 | 6 | 3 | 0 | | |
| G | 18 | 10 | 8 | 0 | | |
| H | 16 | 14 | 2 | 0 | | |
| I | 18 | 6 | 10 | 2 | 32 | 4 |
| J | 16 | 10 | 4 | 2 | 8 | 3 |
| K | 16 | 14 | 2 | 0 | | |
| | 192 | 139 | 47 | 6 | 49 | 10 |

If ♀♀ D and F are disregarded as not adequately progeny-tested, there remain 9 gonadic mosaics each of which had at least 16 examined daughters. Of these, 4 had again one or two gonadic mosaics among their progeny. For two reasons, this figure probably represents an underestimate: (1) Tests on a larger scale might have revealed gonadic mosaics even among the progeny of those ♀♀ which on the present evidence produced only two types of daughters, (2) Only a small proportion of $F_2$ ♀♀ were tested by breeding from their individual daughters. As in the case of the $F_1$ ♀♀, mainly those ♀♀ were selected for a thorough progeny testing/-

testing in which a striking shortage of ♂♂ pointed to the possibility of gonadic mosaicism, and - as pointed out above - this kind of selection will tend to miss gonadic mosaics in which only a relatively small portion of the ovary carries the lethal.

The crucial point for the interpretation of this apparent transmission of mosaicism is the following: were mother and daughter gonadic mosaics for the same mutation, or was mosaicism in the daughter due to spontaneous origin of a new mutation? For ♀ B this point could not be decided on account of inversions in the treated X-chromosome which prevented location of the lethal. For ♀ I identity of the mutation (a semi-lethal) in the mother and 2 of her daughters could be established on account of a very characteristic action of the gene on pupal development. The lethals which occurred in ♀ E and one of her daughters were subjected to location tests and found to be situated at practically the same locus; thus they were presumably identical. The case of ♀ J was complicated and requires description.

♀ J was the daughter of a treated wild-type ♂ and a ClB/scar ♀. Her genotype was thus ClB/treated +. Mated to a $\underline{sc^{Sl}}$ - InS $\underline{w^a\ sc^8}$ ♂ she produced 124 daughters with either Bar or non-Bar eyes, and 11 sons with garnet-coloured eyes. The eye colour of the sons was subsequently shown to be due to an allelomorph of garnet. 16 of her non-Bar daughters, which thus were of the constitution $\underline{w^{a(x)}}$/treated +, were tested by mating them to $\underline{w^a}$ ♂♂.

The breeding tests carried out on these 16 $F_2$ ♀♀ showed them to fall into 4 genotypically distinct classes.

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(x) In the following description the symbol $\underline{w^a}$ will be used to denote the chromosome $\underline{sc^{Sl}\ Ins\ w^a\ sc^8}$

(1) The first class consisted of 3 ♀♀ which produced equal proportions of $\underline{w^a}$ and $\underline{g}$ sons. They thus carried on the treated X chromosome the mutation to garnet which also was apparent in their brothers. 4 of their daughters, when tested, proved likewise to carry garnet.

(2) The second class of $F_2$ ♀♀ consisted of 10 ♀♀ which produced only $\underline{w^a}$ sons. Thus they carried a lethal on the treated X-chromosome. This is in good agreement with the strikingly small number of their brothers (11 as against 124 ♀♀, see above). The lethal was not allelomorphic with garnet and separated from it by several crossing-over units. The location tests gave no indication of a gross structural re-arrangement on the treated chromosome. In the following generation, the lethal bred true in 11 pair-matings and several mass-matings. It was not affected by outcrossing and thus not due to complementary action with an autosomal gene.

(3) The third class of $F_2$ ♀♀ consisted of 1 ♀ only who among her progeny had a normal proportion of wild-type sons. Thus she had received from ♀ J a treated X-chromosome which carried neither the mutation to garnet nor the lethal, each of which were present in some of her sibs, 7 of her daughters were tested and found likewise to carry an unmutated wild-type X.

(4) The fourth and most interesting group of $F_2$ ♀♀ consisted of 2 ♀♀ which bred in a manner similar to that of their mother. Each of these 2 ♀♀ had, in addition to the $\underline{w^a}$ sons with the untreated X, 2 types of sons; garnet and wild-type. Of 8 tested daughters 5 were found to be heterozygotes for a lethal, and 3 heterozygotes for a normal wild-type X-chromosome.

Thus ♀ J and two of her daughters transmitted to their offspring three different types of treated X-chromosome: one carrying garnet, one carrying a lethal, and one without mutation. They appear to have been triple gonadic mosaics, one portion of the ovary carrying the unmutated X, another garnet, and a third/

third the lethal. There may of course have been overlapping between the latter two portions, garnets having been present on some of the lethal-bearing chromosomes. This possibility has not been adequately examined: in two cases in which ♀♀ heterozygous for the lethal were mated to garnet ♂♂ it could be shown not to apply. In Table 6 the pedigree of ♀ J is summarized on the basis of triple gonadic mosaicism of ♀ J and 2 of her daughters, a semi-colon between two gene symbols indicating that the chromosomes carrying these genes occur in different portions of the ovary.

Table 6

Pedigree of a triple gonadic mosaic, ♀ J (Table 5).

$P_1$ treated ♂ + x ClB/scar

$F_1$ (♀ J) ♀ $\frac{ClB}{g;\ l;\ +}$ x ♂ $w^a$

| $F_2$ , | Class 1 | Class 2 | Class 3 | Class 4 |
|---|---|---|---|---|
| ♂♂ | 11 g | 0 | 0 | |
| tested non-Bar ♀♀ (mated to ♂♂ $w^a$) | 3 $\frac{w^a}{g}$ | 10 $\frac{w^a}{l}$ | 1 $\frac{w^a}{+}$ | 2 $\frac{w^a}{g;\ l:\ +}$ |
| $\frac{F_3}{♂♂}$ | 50% $w^a$, 50% g | all $w^a$ | 50% $w^a$, 50% + | 37 $w^a$, 10 g, 4 + |
| tested non-$w^a$ ♀♀ | $\frac{4w^a}{g}$ | 11 $\frac{w^a}{l}$ | $\frac{7\ w^a}{+}$ | $\frac{5\ w^a}{l}$ , $\frac{3\ w^a}{+}$ |

Note: $w^a$ = $sc^{Sl}$ InS $w^a$ $sc^8$ , g = garnet, l = lethal, + = X-chromosome with neither g nor l.

Location of the lethal was carried out only once in $F_2$. Therefore, although it is highly probable that the lethal obtained in $F_3$ was identical with the/-

the original one, this has not been actually proved. However, there definitely was a re-occurrence of an identical visible mutation, garnet, in two daughters of the original gonadic mosaic. This, then, is the third case - out of 4 - in which identity of the recurring mutation in mother and daughters could be established.

The only gonadic mosaic, which had been obtained in the progeny of irradiated ♂♂, showed a similar history of recurring gonadic mosaicism among her progeny, but with the emphasis on a reversion from complete lethality to viability. Among her 6 daughters there was one which appeared to be heterozygous for a full lethal. However, when her progeny were mass cultured together, they produced good proportions of sons with the treated X-chromosome. One daughter, tested by pair-mating, was a gonadic mosaic for a lethal. It is not possible to decide whether this case of apparent reversion of a lethal to its normal allelomorph was due to an induced or spontaneous unstable mutation similar to the unstable genes in Drosophila virilis (Demerec 1941), or to some kind of complementary gene interaction.

## Discussion

In the first part of this paper it has been shown that in the $F_3$ of mustard gas treated ♂♂ the frequency of mutations is significantly increased over that in the controls. Taken on its face value, this finding seems to indicate an after-effect of the treatment leading to a mutation in the generation following the treated one. However, closer analysis revealed that the high mutation frequency in $F_3$ was due to the presence in $F_1$ of a few ♀♀ which were gonadic mosaics for a lethal, i.e. which carried a lethal in part of their ovaries and transmitted it to part of their progeny. The presence of mosaics in the progeny of treated ♂♂ is by itself no proof of a deferred effect of the treatment. It is a well known/-

known fact that also after X-radiation of mature spermatozoa chromosome breaks, which have arisen through immediate action of the treatment, may result in flies which are mosaic for an induced aberration (for a discussion of the probably mechanism underlying this type of mosaicism see Muller 1940). However, when such X-ray "fractionals" have been analyzed by breeding tests (Neuhaus 1935, Pontecorvo 1940) it has been found that in the great majority of cases the gonads contained one kind of tissue only, either mutated or unmutated (see Table 4, first row). If, as is generally assumed, X-ray mosaics arise at the first cleavage division, the scarcity of mosaics with mixed gonads among them may be taken to indicate that in the great majority of cases the whole of the future gonad is derived from one of the first two cleavage cells. If this cell carries the mutation, the resulting mosaic will correspond to type c in Figure 2. If, on the other hand, it does not carry the mutation, a mosaic of type b will be produced. The rare cases of X-ray induced gonadic mosaics (type a, Figure 2), if they cannot be attributed to rare occurrences of a delayed action of irradiation, must then be the result of an infrequent type of first cleavage division by which the material of the future gonads is distributed into both first cleavage cells.

If, therefore, it can be shown that among chemically produced mosaics those with mixed gonads are relatively more frequent than after irradiation, it seems plausible to attribute the excess to cases in which mosaicism arose at a later stage than after irradiation, thus later than at least the first cleavage division. An analysis of apparent semi-lethals revealed that the frequency of gonadic mosaics among them is high, when the fathers had been treated chemically, and negligible when they had been irradiated. It remained to be seen whether this was simply due to the fact that in general mosaics are much more frequent in the progeny of chemically treated than of irradiated ♂♂ (Auerbach and Robson 1946, Auerbach, 1946), or whether there really was an excess of gonadic mosaics among/-

among all mosaics produced by chemical means. This, owing to difficulties inherent in the material chosen (lethals), could not be established by direct observation; but estimates of the ratio of gonadic mosaics to mosaics with uniform gonads after either kind of treatment strongly suggest that this ratio is markedly higher after chemical treatment. This, then, was interpreted to indicate a delayed effect of the chemical treatment in the sense that an initially created instability may lead to an effective mutation at a later division than the one following treatment.

This interpretation was confirmed by the results of tests reported in the third part of the paper. In 4 cases it was found that among the daughters of a gonadically mosaic ♀ there occurred one or more ♀♀ which in their turn were gonadic mosaics for a mutation. Quite possibly breeding tests on a larger scale might have revealed a transmission of the mosaic condition also in some of the remaining cases. In 3 of the 4 positive cases the identity of the mutation in mother and daughters could be established. Here, then, a chemically produced, localized instability on a treated chromosome has been carried through all the cell divisions which separate the fertilized ovum of one generation from that of the next before giving rise to a mutation.

It is noteworthy that gonadic mosaics in the $F_2$ of treated ♂♂ occurred exclusively among the daughters of $F_1$ ♀♀ which themselves had been gonadic mosaics. This is illustrated in Figure 3 in which the various possible female lines originating from treated fathers are represented. No lethal was obtained in over 1000 chromosomes from $F_2$ ♀♀ whose mothers had neither been heterozygotes for a lethal nor gonadic mosaics for a lethal (line A). On the other hand, in no case has reversion of a chemically induced lethal been observed, although ♀♀ heterozygous for such lethals have been mass-cultured very often and sometimes

for/-

for a great number of generations. This is true not only for lethals which occurred at first in a heterozygous $F_1$ ♀ (line B), but equally well for those lethals which at first occurred in a gonadic mosaic (line C) and in certain lines gave repeated origin to mosaics: once such a lethal had established itself in a heterozygous ♀, sister or daughter of a gonadic mosaic for the same lethal, it remained perfectly stable in subsequent generations of this line (line Cb). The facts that stable lines for both the lethal (line Cb) and its normal allelomorph (line Ca) may be derived from a ♀ which is mosaic for them and hands on the mosaic condition to some of her daughters, suggests that a chemically produced instability which does not lead to at least one mutation during the life cycle of an individual has become stabilized. It should be pointed out that the tests on $F_3$ daughters of gonadic mosaics in $F_2$ (line Cc) were carried out on too small a scale (see Table 5) to decide whether or not the mosaic condition might not have occurred also in this generation.

The question arises as to the nature of the initially produced instability. Is it a mutation with a tendency to revert to its normal allelomorph, or is a tendency, induced at a specific locus, to produce a mutation? In the first case, a ♀ which is a gonadic mosaic for a lethal would have started development as a heterozygote for the lethal; but in a portion of her ovary reversion of the lethal to its normal allelomorph would have taken place. With other words, mustard gas would have produced an unstable gene, similar to those found by Demerec (1941) in Drosophila virilis. In the second case, a ♀ who started development without a lethal would have become a gonadic mosaic through a subsequent mutation on the affected chromosome.

The available data do not allow of a definite decision between these two alternatives. Since all 17 gonadic mosaics for a lethal were either direct des-

descendants/-

descendants of treated ♂♂, or daughters of ♀♀ which in their turn had been gonadic mosaics (Fig.3, line C), it is not possible to say whether they started life as heterozygotes for the lethal, or whether the lethal developed in their germ track. At first sight, Table 5 seems to be in favour of the first alternative. Out of 192 tested daughters of gonadic mosaics in $F_1$, 139 carried the lethal as against 47 which were free of it, and a similar disproportion appears among the daughters of gonadic mosaics in $F_2$. Indeed, out of altogether 17 gonadic mosaics, there were only 3 which did not transmit the lethal to the majority of their daughters. This is what one would expect if the lethal had arisen through reversion in an ovary which originally was heterozygous for it. However, it can easily be seen that the same preponderance of lethal-bearing chromosomes is to be expected if the chance for a lethal to develop de novo at an affected locus were sufficiently high to result in more than one mutation during the cell divisions which take place in the germ track. This condition will often be fulfilled for instabilities which, as has been shown above, never seem to skip a generation without at least one mutation taking place. Moreover, selection of ♀♀ for breeding tests was such that only those gonadic mosaics could be detected in which a large portion of the ovary carried the mutation.

In the case of mutations with visible effects a decision as to the origin of a mosaic should be easier to arrive at. An individual which starts with a mutated chromosome is likely to show the effect of the mutation over the greater part of its body, whereas an individual in which the mutation occurs during development will usually exhibit it over less than half of its body or - if more than one mutation took place - in several small, separate areas. Among visible mosaics in the progeny of mustard gas treated ♂♂ there never occurred one in

which/-

which more than one half of the body showed the aberration; on the contrary, in almost one third of 75 carefully examined mosaics for Minute less than one quarter of the body surface showed the aberrant type of bristle (Auerbach 1946). Considering the irregular cleavage pattern of Drosophila (Parks, 1936), such small areas may still have arisen at the first cleavage division and not through delayed action of the treatment, but on the other hand it is highly improbable that this kind of distribution of mutated and unmutated areas would arise through reversion to normal in an originally mutated flies. Thus the data on visible mosaics give no support to the assumption that mustard gas tends to induce mutations which have a tendency to revert to the normal allelomorph.

Also from a purely theoretical point of view, delayed mutation seems a more satisfactory explanation of the observed gonadic mosaics than unstable mutation with a tendency to revert to the normal allelomorph. If the unstable gene is visualized as a gene in which, owing to the chemical treatment, the condition of chemical balance has been altered so that there exists a tendency to revert to the original, stable equilibrium, it is difficult to understand why the same mutation behaves perfectly stable in the majority of closely related lines. It may be objected that in these stable lines the original unstable lethal has mutated once more into a new stable lethal, since a phenotypical distinction between two lethals can usually not be made without embryological investigations. However, in two of the here described cases phenotypical identity of the mutation in stable and unstable lines could be established: one is the garnet mutation in ♀ J, Table 5, the second the characteristic semi-lethal in ♀ I, Table 5.

The observation that stable lines for both the mutation and its normal allelomorph could be established from gonadic mosaics is best explained by the assumption that the primary instability is somehow poised between two stable equilibria: the

original/-

original one of the normal allele, and the new one of the mutation. The fact that in the analyzed cases stabilization occurred preferentially in the direction of the mutation may be an incidental result of the manner in which gonadic mosaics were selected for analysis. This interpretation would also fit a case of visible mosaicism after chemical treatment which has been more fully discussed elsewhere (Auerbach, ~~in press~~ 1946). It is the occurrence among the sons of treated wild-type ♂♂ and attached-X ♀♀ of a ♂ who both in his eyes and his testes was a mosaic for two different allelomorphs at the white locus. Both alleles behaved as stable mutations in further breeding tests. Although alternative explanations can be imagined, it seems at least possible that the primary effect of the treatment consisted in the creation at the white locus of an instability which was poised between the two equilibria represented by the two stable allelomorphs. The fact that none of the tested spermatozoa contained an unmutated X-chromosome is easily explained by the assumption that the primary change was too labile to persist through all the cell divisions preceding sperm formation.

Two similar mosaics for different allelomorphs at the same locus have been found among sons of X-radiated ♂♂ (Neuhaus, 1935, Panshin, 1935). If they are interpreted in the same way, they might be taken to suggest that X-rays, too, may induce primary instabilities which later tend to become stabilized through mutation. Apart from these two isolated and not really well understood cases, X-rays have never been reported to induce instabilities of the type described here, nor unstable genes like those described by Demerec, all of which arose spontaneously. This is true in spite of the fact that the number of observed X-ray mutations is infinitely greater than of those which occurred spontaneously or after chemical treatment. This might conceivably be due to the difference in the amounts of energy supplied by chemical reactions on the one hand, hard X-rays such/-

such as are used generally for mutation work on the other hand. Whereas the energy supplied by the impact of X-rays will in the vast majority of cases be sufficient to lift the gene from one stable equilibrium into another - also relatively stable - one, the limited quantity of energy which is supplied by a chemical reaction may sometimes just be sufficient to bring about transformation into an intermediate labile state, the final attainment of a new stable condition or the sliding back into the old one being achieved subsequently under the influence of random temperature oscillations.

If, as appears possible, a proportion at least of the so-called spontaneous mutations arise through the action of naturally occurring mutagenic substances (Auerbach and Robson 1944), localized chromosome instabilities might be expected to occur also without special treatment. Possibly unstable mutations have arisen in this manner. Another example to the point is Castle's (1929) line of three generations of mosaic rabbits. Perhaps some of the outbreaks of specific mutations in certain stocks (e.g. Goldschmidt 1939, Mampell 1943) may have been caused in a similar way. It is interesting to speculate whether also in organisms other than Drosophila certain types of semi-sterility may be caused by gonadic mosaicism for lethal or semi-lethal genes.

Acknowledgment

The author is much indebted to Mr Condon, Mr M.Y.Ansari, and Dr M. Ginsberg for carrying out the exposures to mustard gas.

Summary

The frequency of sex-linked lethals in the $F_3$ of mustard gas treated ♂♂ was significantly higher than in the controls, when the $F_3$ was derived from daughters of the treated ♂♂. This was shown to be due to the presence in the $F_1$ of ♀♀ which were gonadic mosaics for a lethal, i.e. which carried a lethal in only

a/-

a part of their ovaries.

A high proportion of apparent semi-lethals in the progeny of chemically treated, but not of irradiated ♂♂ was in reality due to gonadic mosaicism of $F_1$ ♀♀. It was estimated that in the progeny of chemically treated ♂♂ gonadic mosaics form a higher proportion of all mosaics than in the progeny of irradiated ♂♂. This is taken to indicate that the effect of mustard gas may lead to mutation at a division later than the one following treatment.

In several cases, daughters of chemically produced gonadic mosaics were again gonadic mosaics for the same mutation: in these cases, an induced chromosomal instability must have been carried through many cell generations before giving rise to a mutation. In the majority of lines derived from gonadic mosaics, both the lethal and its normal allelomorph behaved perfectly stable.

It is suggested that the primary effect of mustard gas and similar chemicals may sometimes consist in the creation of an instability which may become stabilized in two different ways: either by reverting to the old equilibrium of the normal allelomorph or by attaining the new equklibrium of a stable mutation.

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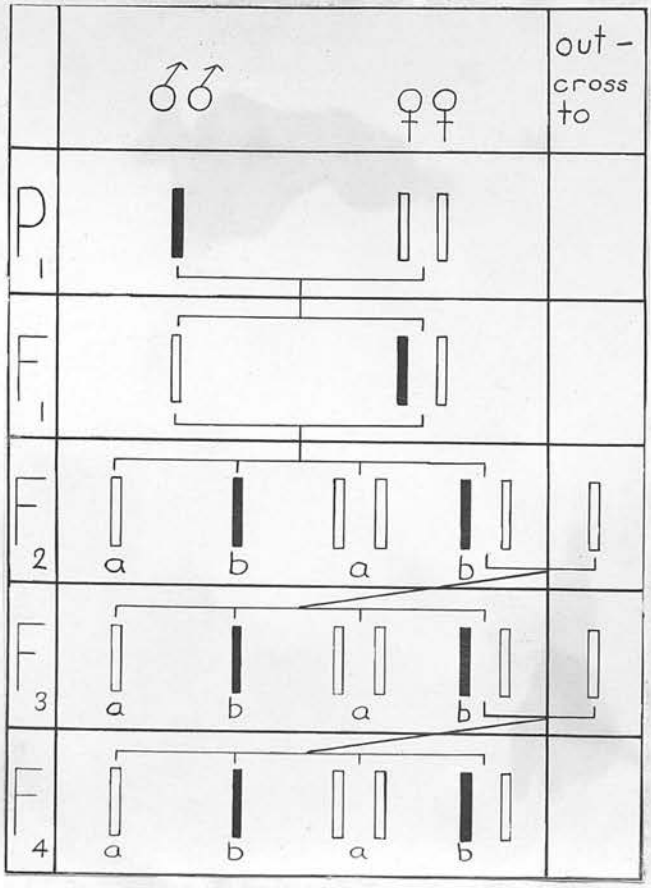


Fig. 1.

Scheme of matings for the detection of a delayed effect of chemical treatment.

▮ = treated, ▯ = untreated X-chromosome.

♀♀ used for the outcrosses in $F_2$ and $F_3$ were chosen from cultures in which both types of ♂♂ were present.

A lethal which has arisen in the treated X-chromosome becomes manifest as absence of ♂♂ of type b

in $F_2$, if the lethal arose in $P_1$;

in $F_3$, if the lethal arose in $F_1$;

in $F_4$, if the lethal arose in $F_2$.

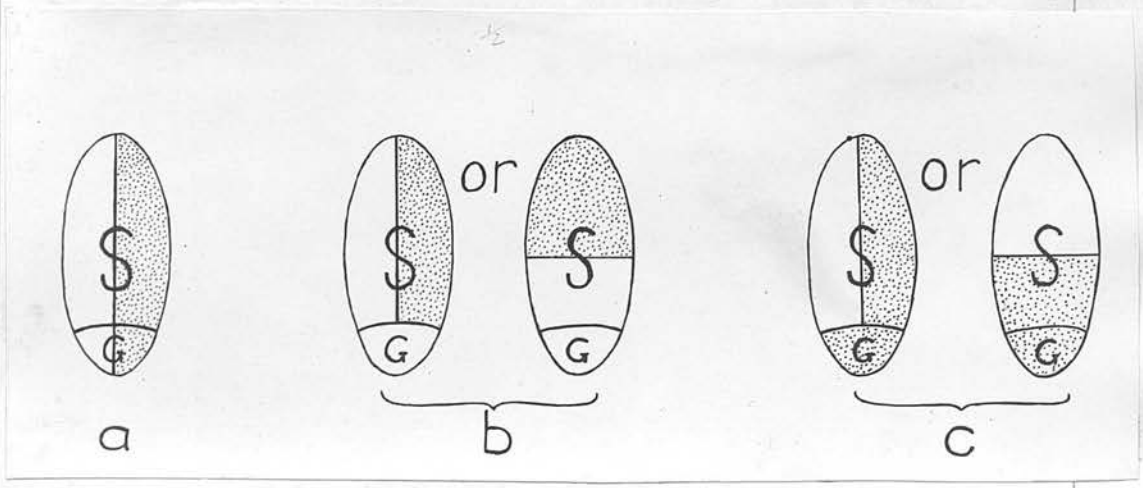


Fig. 2.

Types of mosaics for a lethal.

a = gonadic mosaics  b and c = gonosomic mosaics

stippled = tissue heterozygous for a lethal.

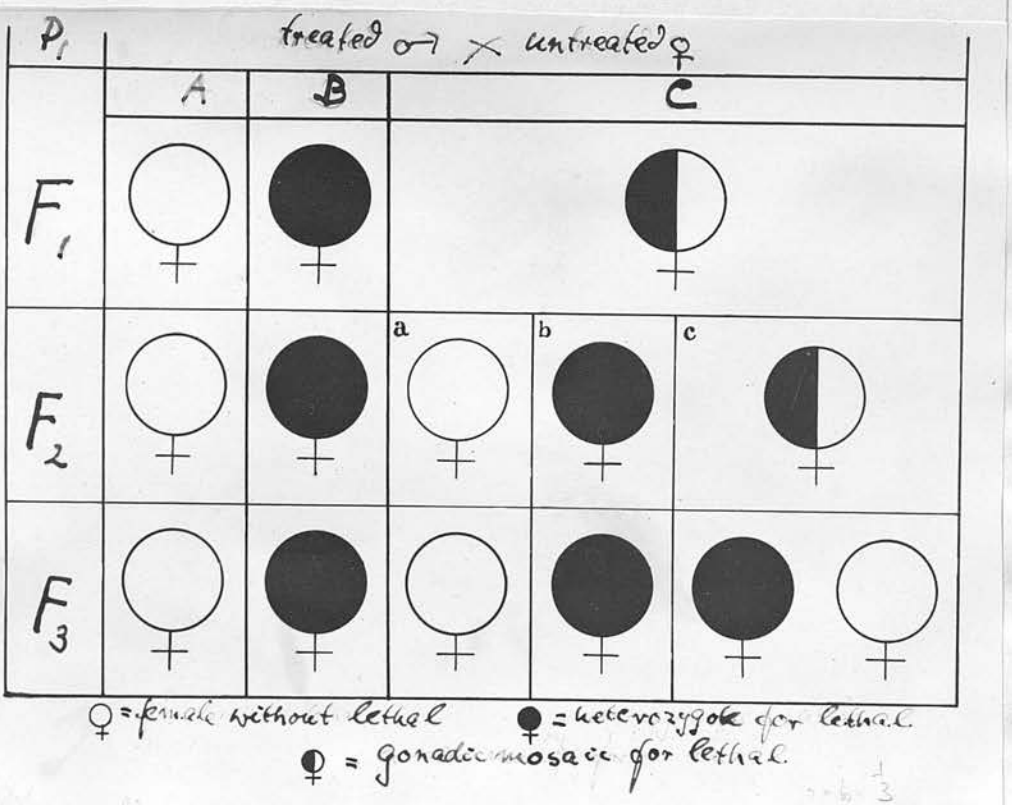


Fig. 3.

The three types of female lines derived from treated males.

# ABNORMAL SEGREGATION AFTER CHEMICAL TREATMENT OF DROSOPHILA

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AMONG the mustard gas compounds which have been shown to possess a high mutagenic capacity combined with the ability to produce chromosome breaks, N-methyl di-(2-chloroethyl) amine, $CH_3 \cdot N(CH_2CH_2Cl)_2$, is one of the most efficient (AUERBACH and ROBSON, in press). In an experiment carried out in 1943, in which wild type males of *Drosophila melanogaster* were exposed to vapors of this substance, there occurred an abnormal and inherited type of segregation which points to an effect of the chemical on the centromere of the treated X chromosome.

The treated males were mated to *ClB/scar* females.[1] One of the Bar daughters from this mating, when mated to a *scar* brother, produced a highly unusual type of progeny. If *t* is used to indicate the treated X chromosome, the data may be summarized as follows:

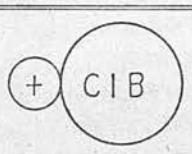
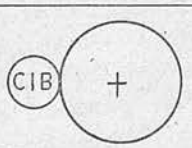
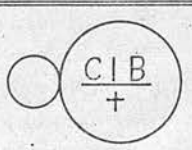
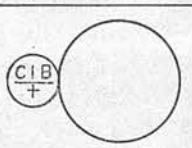
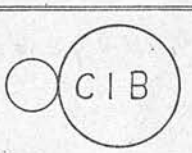
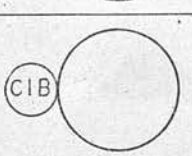
| | | |
|---|---|---|
| $P_1$ | *ClB/scar* ♀ ♀ × *t* ♂ ♂ | |
| $F_1$ | *ClB/t* ♀ × *scar* brother | |
| $F_2$ phenotypes | ♀ ♀ | ♂ ♂ |
| | 36 *sc v B* | 34 *scar* |
| | 11 wild | 3 wild |
| | 9 *B* | 1 *sc* |
| | 1 *sc B* | |
| | 57 | 38 |

All wild-type males, the *sc* male, and eight tested *scar* males proved to be sterile. The other *scar* males were not tested, but it may be presumed that they, too, were sterile. Sterility of the wild type males may have been caused by a mutation on the treated X chromosome. The same applies to the *sc* male which must have been a crossover between *ClB* and *t*. Sterility of the *scar* males, on the other hand, points to their having lacked a Y chromosome, and thus having been produced by primary nondisjunction in the *ClB/t* female.

Such a high degree of primary nondisjunction is quite unusual. From a study of the segregation data there emerge several points which allow inferences on meiosis in the *ClB/t* female. As is usual with primary nondisjunction, there are

[1] *scar* = *sc*(scute), *v*(vermilion), *f*(forked), *car*(carnation). It may be recalled that the *ClB* chromosome contains the mutant genes *sc*(scute), $t^2$(tan$^2$), *v*(vermilion), *sl*(small wing) and *B*(Bar eye).

considerably more exceptional XO males (*scar*) than exceptional XXY females (Bar). This is attributable to the fact that nullo-X eggs, from which the non-disjunctional males arise, may be produced not only by nondisjunction carried to the final result of two X chromosomes becoming included into the same cell,

| Type of segregation | | Ovule and polar body | Zygote after fertilization by sperm | |
|---|---|---|---|---|
| | | | *scar* | ~~X~~Y |
| A | 1 |   | *sc v B* ♀ 11 (+\|*sc B*) | — |
| | 2 |   | wild ♀ 11 | wild ♂ 3 (+\|*sc*) |
| B | 1 |   | — | *B* ♀ 9 |
| | 2 |   | *scar* ♂ 9 | — |
| C | 1 |   | *sc v B* ♀ 25 | — |
| | 2 |   | *scar* ♂ 25 | — |

A-normal segregation. B-non disjunction. C-loss of treated X.

but also by loss of one X chromosome on the spindle. The observed data allow an estimate to be formed of the relative frequencies with which the treated X chromosome (A) segregates normally from the *ClB*, (B) fails to disjoin from the *ClB* and is included with it into the same cell, ovum or polar body, and (C) is lost on the spindle. Figure 1 shows these possibilities in a diagrammatic way.

There are nine Bar females resulting from the inclusion of both X chromosomes into the ovum (B, 1). It may be presumed that there is a corresponding number of nullo-X eggs produced through the inclusion of both X chromosomes in the polar body (B, 2). This, then, accounts for nine out of the 34 exceptional males. The remaining 25 must be due to a combination of segregation of the *ClB* into the polar body and loss of *t* on the spindle (C, 2). This, however, represents only half of the cases of loss of *t;* where this loss is combined with segregation of *ClB* into the ovum, the resulting fly will be a *sc v B* female, (C, 1). Thus there are about 50 cases in which the treated X is lost on the spindle as against about 18 in which it follows the untreated one into the same cell: loss of the treated X is about $2\frac{1}{2}$ times as frequent as nondisjunction.

When the 25 *sc v B* females which may be presumed to have arisen through loss of *t*(C, 1) are subtracted from the total of 36, there remain 11 which arose through normal segregation, corresponding to the 11 wild-type (*scar/t*) females. (A, 1, 2). The shortage of wild type females, like their sterility, possibly was due to mutation on the treated X chromosome. Comparison of A and B shows that normal segregation was about equally as frequent as nondisjunction. This accounts for the two most striking features of the segregation data: the similar frequencies of nondisjunctional males and *sc v B* females, and of nondisjunctional females and wild type females.

Five of the nondisjunctional Bar females of the genotype *ClB/t/Y* were crossed to males *sc v delta 49 od car*.[2] All of them produced mainly nondisjunctional sons and *sc v B* daughters and very little else. The combined data are as follows:

*ClB/t/Y* ♀ ♀ × *sc v delta 49 od car* ♂ ♂

| daughters | sons |
|---|---|
| 77 *sc v B* | 80 *sc v od car* |
| 7 wild | 3 wild |
| 6 *B* | |
| 90 | 83 |

This segregation shows the same features as the one observed in $F_2$, namely a degree of nondisjunction which is unusual even for secondary nondisjunction in the presence of an inversion, and numerical similarity of patroclinous males with *sc v B* females, and of nondisjunctional B females with wild type females. The only difference from the $F_2$ is a shift of the frequencies in favor of exceptional progeny, and this is what would be expected from the influence of the extra Y chromosome on meiosis. A similar calculation as has been carried out above gives the following frequencies for the three initial types of segregation: (A) normal segregation: 12, (B) nondisjunction: 14, (C) loss of *t* on the spindle: 174. It will be seen that there has been no relative increase in the frequency with which the treated X chromosome fails to disjoin from the untreated one, but that the relative frequency of its loss has been increased about five times.

[2] delta 49=large inversion, *od* (outstretched).

Data obtained with breeding from *scar/t* females were of the same type, but too small for numerical interpretation. One out of six mated females differed from all others in producing a progeny with a normal proportion of wild type sons and containing no nondisjunctional individuals. Apparently this female had lost both the semi-lethal and the condition responsible for nondisjunction.

Crossing over took place in all regions separated by the four marker genes of the *sc v f car* chromosome; this shows that no gross rearrangement was present. This was confirmed by an examination of salivary slides kindly carried out by DR. SLIZYNSKI. There appeared to be an abnormality in the region of the centromere, but its nature could not be determined on the basis of the available slides.

Owing to its peculiar behavior the treated X chromosome was rapidly lost: through inviability and sterility of the males carrying it, through loss on the spindle, and through crossing over.

## INTERPRETATION

The treated X chromosome was characterized by four peculiarities: it carried a semilethal, it carried a sterility mutation, it had a tendency to follow the untreated X chromosome into the same cell at meiosis, and it had a still greater tendency to be lost at meiosis. The viability and sterility mutation possibly were identical, moreover they must have been located in the same region which carried the cause of the abnormal segregation, for all four peculiarities were lost simultaneously from one female. This region appears to have been the centromere region. It may be considered that under the influence of the chemical treatment a change took place in this region—either a small rearrangement involving the centromere, or a "mutation" of the centromere itself—as a consequence of which the treated chromosome tended to follow its homologue at meiosis on the spindle, either the whole way with inclusion of both in the same nucleus, or part of the way with subsequent loss on the spindle. The presence of an extra Y chromosome does not seem to have influenced the relative frequency of nondisjunction as compared with normal segregation; but presumably through competition for pairing, it appears to have created additional opportunities for loss of the treated X.

Crossing over between the treated X and the *ClB* chromosome seems unusually high: when the nondisjunctional sons, which obviously cannot have received a crossover chromosome, are disregarded, there remain 150 flies in $F_2$ and $F_3$ together, and two of these have crossover chromosomes (one single, the *sc* male, and one double, the *sc B* female). The possibility may be considered that crossing over was facilitated through weakening of the centromere.

If the treated X chromosome were lost also during the mitotic divisions, this should lead to a frequent occurrence of male spots in females heterozygous for it. Actually, only one gynandromorph was found among over 50 females which were heterozygous for the treated chromosome. Apparently disturbances of mitosis, if they occurred at all, were infrequent. This suggests that the erratic behavior at meiosis was caused by the unequal strength of the treated centromere and the homologous one. In mitosis, the two centromeres which separate

from one another are the daughters of the same original centromere and must be supposed to be equal in strength independently of whether the mother centromere was normal or abnormal.

Several other cases of abnormal segregation were observed after chemical treatment, but none of them was sufficiently analyzed to suggest the underlying cause. The interest of the present case lies in the fact that it points to the possibility of chemical effects on the centromere. This is in keeping with unpublished results of DR. KOLLER who found definite effects of mustard gas on the centromeres of *Tradescantia* chromosomes.

ANDERSON (1929) has described a line of exceptionally high primary and secondary nondisjunction in *Drosophila melanogaster*. However, the percentages of nondisjunction recorded by him are very much lower than the present ones, and there was no loss of the involved X chromosome on the spindle. This indicates already that the centromere was not responsible, and as a matter-of-fact, ANDERSON was able to locate the cause for the abnormality in the neighborhood of the *v* locus, that is well away from the centromere. Thus, his case seems hardly comparable with the one described here.

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# THE CHEMICAL PRODUCTION OF MUTATIONS

by

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A genetical mutation is a change, presumably chemical in nature, in one of the genes which compose the chromosome thread. The mutated gene is as stable as the original one was before; it goes on reproducing replicas of its mutated self and thus initiates a new hereditary line. It is believed that without mutation life would never have proceeded beyond its very first elementary beginnings. Yet the mechanism of this important process is practically unknown. With the discovery by H.J.Muller in 1927[1] that mutations can be produced artificially by X-rays a new approach to the problem of mutation was opened up. A great number of new facts relating to this problem were brought to light. One of the most important results was the discovery that the term "mutation" includes a number of distinct processes. In addition to gene mutation as defined above, X-rays produce breaks in the chromosome threads; when the resulting fragments join up into novel combinations, so-called chromosome re-arrangements, hereditary changes closely resembling mutations may be produced. Rules connecting quantity and quality of radiation with type and frequency of mutations were discovered, and inferences could be drawn on number of ionisations required, size of the sensitive gene volume, etc.[2] Yet the actual processes of gene mutation and chromosome breakage - if indeed these are essentially different from one another - are still as mysterious as ever.

X-rays are destructive and non-discriminating. It is conceivable that less severe methods of producing mutations might make possible a closer insight into the processes concerned. An advance in this direction was made when it

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was found that ultraviolet light, too, is capable of producing mutations. The restriction of the effective wave length to a comparatively narrow range which includes the absorption bands of nucleic acid and certain protein components supported the hypothesis, put forward by radiation geneticists, that the first step towards the production of a mutation consists in the absorption of an energy quantum by some constituent of the chromosome. But the hope that more specific effects, dependent on the wave length, might be produced, was not realised. Certain differences between the action of ultraviolet and that of the much shorter waves of X- and gamma-rays are not yet understood, but may eventually prove helpful in the analysis of the mutation process. (3)

Chemical substances with mutagenic properties should be particularly useful tools for attacking the problems of mutation. If, as we assume, a mutation is a chemical process, then knowledge of the reagents which are capable of initiating this process should throw light not only on the reaction itself, but also on the nature of the gene, which is the other partner in the reaction. Moreover, it could be hoped that among chemical mutagens there might be some with particular affinities for individual genes. Detection of such substances would not only be of high theoretical interest; it would also open up the long sought-for way to the production of directed mutations.

The search for chemical mutagens has been going on for well over 20 years. The choice of substances tried for the purpose was mainly random. Iodine, ammonia, metal compounds, carcinogens, are only some out of the great number tested. Results were often clearly negative, and no clearly positive ones had been obtained up to the beginning of our experiments in 1940 (for references see

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(4), (5), (6). It is obvious that a chemical mutagen must possess very special properties. It must be able to act selectively on the genic material without at the same time destroying the cell which contains this material. It was, therefore, only to be expected that many substances would have to be tried before an effective one was found, and the search was continued by many workers. This search was encouraged by the accumulation of data which pointed to an influence of physiological conditions such as age (7), sex (5), starvation (8), and of the genotype itself (9, 10, 11) on mutation rate. If, thus, chemical conditions created by the organism itself are capable of influencing the process of mutation, it did not seem beyond hope that chemical substances introduced from outside might have similar effects.

The choice of mustard gas for trials of this kind was suggested by observations which pointed to its interference with cell division. Mustard gas burns, like X-ray burns, heal only with difficulty, and even after they appear to have healed they have a tendency to break down again. In addition it was found that vaginal epithelium of an ovariectomized mouse which has been exposed to a weak solution of mustard gas fails to manifest the mitotic activity which normally follows stimulation with oestrogens, and that this inhibition of mitosis lasts for several weeks after the exposure to mustard gas. It is well known that the chromosome breaks and re-arrangements caused by X-radiation interfere with cell proliferation, partly through mechanical disturbances of mitosis and partly through death of those cells which, after distribution of the fragments and new chromosome combinations into the daughter cells, do not receive a sufficiently normal set of chromosomes. It was thought possible that mustard gas, like X-rays, may inhibit cell division through direct action on the chromosomes.

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In the autumn of 1940, experiments were started to find out whether mustard gas is capable of producing gene mutations and chromosome re-arrangements. Drosophila melanogaster was used as test animal. The flies were exposed to mustard gas vapor, first in a closed chamber, later in a container through which air mixed with mustard gas was sucked. The first results immediately gave promise of success. Both males and females became sterilized to a degree which depended on the dose. Sterility was found to be due to two independent causes, both of which are also known to be involved in the production of X-ray sterility. Firstly, gametogenesis is inhibited so that after a time no more ova and spermatozoa are available. And, secondly, lethality is high among zygotes from treated eggs and - more important still - among eggs laid by untreated females which have been mated to treated males. Since the spermatozoa do not lose their motility as a result of the treatment, the most likely explanation was that mustard gas, like X-rays, produces chromosome breaks and re-arrangements in the sperm.

In order to obtain conclusive proof that mustard gas exerts an action on the chromosomes, genetical methods for the detection of mutations were applied. Male flies were mated to untreated females, and the progeny ($F_2$) was examined for the occurrence of mutations. Early mutation work, as well as some more recent work on organisms which are genetically less thoroughly known than Drosophila, has suffered from the impossibility of eliminating the large personal error; for a trained worker may spot abnormalities which may pass unnoticed by a less experienced or less observant person. In Drosophila genetics this obstacle has been removed by methods which, in the main, have been designed by H.J.Muller, and without which the quantitative analysis of genetical radiation effects would have been impossible. The essential feature of these methods/-

methods is their restriction to the detection of so-called lethal mutations, i.e. of mutations which are so harmful that they prevent development of the individual. Hence lethal mutations are detected by the absence from the progeny of a whole class of flies, and since presence or absence are characteristics about which any two observers are likely to agree, these methods reduce the personal error to a minimum, while at the same time allowing the study of large samples without excessive labour. Particularly useful for large scale tests are methods like the famous ClB test which are designed to detect sex-linked lethals, i.e. lethals on the sex-chromosome, of which the male has only one, while the female has two. A sex-linked lethal prevents the development of a male carrying it, while it usually does not interfere seriously with development of the female. In the ClB test each treated or control sex-chromosome becomes subsequently represented, in the $F_2$, by a whole culture of flies, and if a lethal has arisen on a sex-chromosome, the corresponding culture will consist entirely of females - a fact which is of course readily observed even by an untrained person.

The first ClB test with mustard gas was carried out in April 1941. Its result was spectacular beyond expectation. Whereas the rate at which sex-linked lethals arise spontaneously in laboratory stocks rarely approaches 1%, 90 lethals were found in about 1300 treated sex-chromosomes. This represents a mutation rate of over 7%. Only 3 sex-linked lethals were found in an equivalent number of untreated chromosomes representing a rate of 0.2%. Similar results had previously been obtained only with X-rays or other high energy radiation. Further tests fully confirmed and even exceeded the first success, and up to 24% lethals were produced. Higher percentages can hardly be expected, because concomitantly with the increase in mutation rate sterility becomes more and more severe.

Genetical/-

Genetical analysis of the lethals produced in the first ClB test indicated that some of them were due to or combined with chromosome re-arrangements, and these genetical findings were confirmed by cytological examination carried out by Dr Slizynski. A special test for the production of chromosome re-arrangements by mustard gas was undertaken in December 1941. The method was designed to spot translocations, i.e. re-arrangements through which two chromosomes have exchanged portions with one another. Spontaneous translocations are so exceedingly rare that the use of controls was not considered necessary. The result left no doubt about the capacity of mustard gas to produce chromosome re-arrangements; 7 translocations were found in 816 treated nuclei. A report on these results was sent to the Ministry of Supply in March 1942, but, like all this work, could not be published owing to the security ban on work with war gases. In subsequent experiments more translocations as well as other types of re-arrangements were produced. Since only Drosophila had been used for all these studies, it was gratifying that cytological investigations on pollen mother cells of Tradescantia carried out by Dr Koller in 1943 fully confirmed our finding that mustard gas can produce chromosome breaks and re-arrangements.

The similarity between the genetical effects of mustard gas and of X-rays are so striking that only gradually did certain differences between the two types of action come to light. Yet special interest attaches just to these differences, because a comparison between chemical and physical mutagens seems a hopeful approach to the problem of mutation. The first difference appeared in the work on translocations. It has been shown that the frequencies of X-ray induced lethals on the one hand, and of X-ray induced translocations on the other, bear a mathematical relationship to the dose administered, the first increasing directly as the dose, the second approximately as its 3/2th power.(2)

Consequently/-

Consequently, for a given dose of X-rays (as measured in roentgen units) there exists a numerical relationship between the numbers of lethals and of translocations produced. Thus, a dose of 3000 r-units produces about 9% sex-linked lethals and about 6% translocations between chromosomes II and III of Drosophila melanogaster. After mustard gas treatment, this relationship is shifted very markedly in favour of sex-linked lethals. Instead of the expected 6% only 0.5% translocations between chromosomes II and III were produced in an experiment in which the rate of sex-linked lethals was 9%, and a similar relative shortage of translocations was observed in subsequent tests. At first sight, these observations seem to indicate that mustard gas is less efficient than X-rays in breaking the chromosome thread. However, it is well to be cautious in drawing this conclusion. It has to be kept in mind that with the methods used we could not detect the primary breaks, but only a proportion of the subsequently formed re-arrangements. It is conceivable that chemical treatment interferes with the process of rejoining of broken ends in such a way that a given number of breaks results in fewer observable re-arrangements than would be formed by the same number of X-ray breaks. Special tests are required to decide this point.

On the other hand, it does not seem as though mustard gas were less efficient than X-rays in the production of very small, so-called "minute" re-arrangements. Slizynski and Slizynska,(12) in a cytological study of sex-linked lethals produced by various agencies, have found that in about 20% of cases the genetical change underlying the production of a lethal is a minute deficiency in the chromosome, and this frequency appears to be the same after X-rays, after ultraviolet radiation, and after mustard gas treatment. These findings emphasize the similarity, often pointed out by geneticists, between true gene mutations and minute/-

minute chromosome re-arrangements, and they do not contradict the possibility that so-called gene mutations may be nothing more than chromosome re-arrangements of so minute a size that they elude detection by cytological methods. It will be of great interest to determine whether small deficiencies form an equally high proportion of lethals which have been produced by less potent chemical substances than mustard gas.

A second difference between the actions of X-rays and of mustard gas came to light in the course of a study of visible mutations after chemical treatment. In one respect this study was disappointing; the mutations observed were of the same types as those found after X-ray treatment, and there was no indication of any specific effects of the gas on individual genes. Mustard gas seems to act as indiscriminately as X-radiation. There is, however, a difference between these two agencies which involves not the types of mutation which they produce, but the way in which the mutations become manifest in the offspring of the treated flies. After X-ray treatment of males most of the mutated offspring show the induced abnormality (such as yellow body colour instead of the normal gray) over the whole surface of their body. Only a small proportion, less than 15%, of the mutated individuals, are mosaics, i.e. show the abnormality in a part of their body, the remainder being normal. In the progeny of mustard gas treated males, on the other hand, mosaics form a high proportion, usually between 30 and 50%, of all mutated individuals.[13] Moreover, whereas the gonads of X-ray mosaics rarely contain both normal and mutated cells, those of mustard gas mosaics quite frequently appear to do so. A special study has been made of such "gonadic mosaicism" in respect of sex-linked mutations.[14] A female, daughter of a treated male, whose ovaries contain a patch of tissue in which the cells carry a sex-linked lethal, will have fewer sons than a normal

female/-

female, the shortage of sons depending on the relative sizes of the normal and mutated portions of the ovary. A similar depression of the sex-ratio occurs also in the progeny of females who carry, evenly distributed through all cells of their ovaries, a sex-linked "semi-lethal" mutation, i.e. a mutation which weakens the males so that only a proportion of them are capable of completing development. Analysis of females giving a low sex-ratio has shown that among daughters of irradiated males this abnormality is almost always due to a semi-lethal mutation affecting the whole of the ovary. On the other hand, in 9 out of 20 daughters of mustard gas treated males, the shortage of sons was due to the presence in their otherwise normal ovaries of a mosaic patch carrying a sex-linked lethal. Finally, mention should also be made of a striking case of mosaicism in which a son of a mustard gas treated male was, both in the gonads and in the soma, a mosaic for two different mutations of the same gene, although it must be assumed that in the treated spermatozoon each treated gene was present only once.

An explanation which seems particularly satisfactory in accounting for all these observations is that the gene affected by treatment does not always mutate at once, but may acquire a tendency to mutate which remains latent until a later cell division. Support for this hypothesis was obtained when it was found in several cases that the offspring of gonadic mosaics for a mutation again were gonadic mosaics for the same mutation. In these cases, an induced specific instability seems to have been transmitted from one generation to the next before giving rise to a stable change. No parallel observations have been reported in literature on radiation genetics; but it seems worth noting that so-called "unstable" genes, i.e. genes which tend to mutate repeatedly in the same direction, have been found several times in untreated material. (15)

The/-

The difference between the mutations produced by short wave radiation and chemical reaction may be tentatively ascribed to the different amounts of energy involved in the two types of reaction. In short wave radiation the energy made available is usually sufficiently large to produce a catastrophic alteration in the structure of the gene, by transforming it from one stable configuration to another. On the other hand, reaction of the gene with a chemical substance, owing to the smaller amount of energy involved, may produce a less drastic effect, by transforming it to an intermediate metastable configuration. Such a configuration will, of course, tend to undergo "spontaneous" alteration to another and more stable configuration, i.e. an "unstable" gene is produced.

After the first positive results with mustard gas the search for chemical mutagens was extended to substances which either in their chemical structure or in their pharmacological action are related to mustard gas. Mustard gas is a fixative of protoplasm with unusual power of penetration. It was soon seen that these two properties by themselves are not sufficient to make a substance mutagenic, for neither osmic acid nor picric acid affected the mutation rate in tests in which the majority of the treated individuals were killed. Neither can it be said that every potent vesicant is a mutagen, for lewisite gave entirely negative results in two ClB tests. So far only three substances have been found which give similar genetical effects to mustard gas. They all belong to the class of nitrogen - or sulphur mustards. Their chemical formulae are:

(1) $O(CH_2.CH_2.S.CH_2.CH_2.Cl)_2$, (2) $N(CH_2.CH_2\ Cl)_3$, and (3) $CH_3.N(CH_2.CH_2\ Cl)_2$.

As the chemical mutagens presumably attack the genic material directly, it was noted with interest that these active compounds all contain an unsaturated atom (:S or :N) which might combine with materials composing the gene, and that this/-

this activity would be enhanced by the type of side-chains present in the vesicant mustards (e.g. Cl $CH_2$.$CH_2$). With this type of structure is associated the tendency to intra-molecular cyclization to form onium compounds, (16) characteristic of the active :N and :S vesicants. Assuming that the mutagenic action is due to the reaction on the unsaturated atom of the uncyclized compound with the gene, it was thought likely that replacement of the :S and :N by :O would not produce active materials, as the addition compounds of divalent oxygen are not stable in aqueous solution (and "O mustard" is not a vesicant). Stable addition compounds are however formed by compounds having the :CO group, and though the corresponding "CO mustards" were not tried, the tear-gases chloracetone and dichloracetone (compounds with Cl $CH_2$ - side chains instead of the $ClCH_2$.$CH_2$ - of mustard gas) were examined. The activity of these compounds was extremely weak; in fact the results were not clearly positive, although they suggest the possibility that both these compounds are slightly mutagenic. By analogy with the vesicant action of the :S compounds, the chlorethylacetones might be more active. It has not yet been possible to test these substances.

Other groups which might replace the :S and :N of the mustards and retain the additive ability of the molecule include :As, although compounds with :As are rather unstable under physiological conditions, and are mostly toxic. The corresponding :P compounds are apparently out of the question in this connection, as they are spontaneously inflammable in air. The :$SO_2$ compounds may similarly be expected to show some activity, but not the :SO ones. This parallels their vesicant action. There is also the possibility of using nitrile, <u>iso</u>nitrile, and the corresponding thiocyanates and <u>iso</u>thiocyanates for the/-

the co-ordinating group, and attention was therefore directed to allyl iso-thiocyanate. A weak, but definite mutagenic activity could be demonstrated for this compound.[17]

On the other hand, this type of chemical structure does not seem a necessary prerequisite for a mutagenic substance. This is shown by the fact that Hadorn and Niggli[18] have obtained considerable numbers of mutations by exposing explanted ovaries of Drosophila to weak solutions of phenol.

Allyl isothiocyanate or mustard oil, just referred to, occurs naturally in plants of the genus Brassica. We do not know whether it acts as a mutagen in these plants, but it is interesting to speculate how far naturally occurring mutagens may be responsible for spontaneous mutability. It has been shown by Muller and Mott-Smith[19] that cosmic radiation and natural radioactivity are quantitatively insufficient to account for the observed rates of spontaneous mutation. Timoféeff-Ressovsky, Zimmer and Delbrück[20] have suggested that random temperature oscillations inside the nuclei may occasionally overstep the energy threshold required for the production of a mutation. In the light of the results reported here it seems, however, possible that a certain proportion of natural mutations may be due to the action of mutagenic substances within the organism, and whose production may itself be the consequence of gene action. Such an assumption finds support in the known cases in which definite genes influence mutability of the rest of the genes or even of a specific gene (11, 13, 12). Search for natural mutagens is therefore of high interest, but may well turn out to be exceedingly difficult; for by its very nature a natural mutagen can have no drastic effect in the species in which it occurs, otherwise the species could not survive. Moreover, a physiological system which includes mutagens whose production is controlled by genes will in the course/-

course of its evolution have attained a finely attuned equilibrium between the strength of the effective substances and the sensitivity of the gene or genes on which they act. Removed from its normal genotypical environment, a mutagen may produce quite different effects or none at all. Therefore, results obtained with one organism may not be transferable to another, in contrast to results gained with such drastic agencies as X-rays and - presumably - mustard gas. Nevertheless it is tempting to consider the possibility that one of the means by which evolution adapts mutability to environmental requirements is the achievement of a balance between the production of mutagens and sensitivity to them.

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